# A COMPARISON OF THE GLYCINE CONTENTS OF THE PROTEINS OF NORMAL AND CHONDRODYSTROPHIC CHICK EMBRYOS AT DIFFERENT STAGES OF DEVELOPMENT

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Patton and Palmer ('36) recently reported that glycine is synthesized during the development of the hen's egg, and that a significant difference is found between the glycine contents of the proteins of normal and chondrodystrophic chick embryos. The present paper is an extension and a substantiation of their work.

Glycine has been determined in sixty normal embryos, and in eighty-three chondrodystrophic embryos. The latter were obtained by Hutt and his associates in the routine examination of about 7000 embryos which died during incubation. The method of Patton ('35) was used, excepting that calculations were made on the basis of dry weight, each sample of four eggs being acetone-alcohol-ether extracted (48 hours each) and dried 24 hours at 100°C.

Table 1 presents evidence of glycine synthesis during development, in both White Leghorn and Barred Plymouth Rock embryos. Triplicate analyses were made. The percentages for Barred Plymouth Rock embryos, from 12 to 21 days, were obtained by adding the separate values for yolks and embryos in table 2. Curves plotted from these data show minimal

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TABLE 1

*Glycine content of normal embryos, entire egg*

| AGE | GLYCINE | | | |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 | Mean |
| | | White Leghorn | | |
| *days* | % | % | % | % |
| 3 | 1.35 | 1.32 | 1.29 | 1.32 |
| 6 | 1.27 | 1.27 | 1.27 | 1.27 |
| 9 | 1.28 | 1.25 | 1.22 | 1.25 |
| 12 | 1.27 | 1.27 | 1.27 | 1.27 |
| 15 | 1.61 | 1.45 | 1.53 | 1.53 |
| 18 | 2.05 | 1.92 | 1.88 | 1.95 |
| 21 | 2.08 | 1.87 | 1.92 | 1.96 |
| | | Barred Plymouth Rock | | |
| 0 | 1.76 | 1.73 | | 1.75 |
| 3 | 1.76 | 1.68 | 1.73 | 1.72 |
| 6 | 1.08 | 0.52 | 0.60 | 0.73 |
| 9 | 1.48 | 0.92 | 1.40 | 1.27 |
| 12 | Summation of yolk and embryo | | | 1.10 |
| 15 | Summation of yolk and embryo | | | 1.56 |
| 18 | Summation of yolk and embryo | | | 1.80 |
| 21 | Summation of yolk and embryo | | | 2.20 |

TABLE 2

*Glycine content of normal Barred Plymouth Rock embryos and yolks*

| AGE | DRY WEIGHT | GLYCINE | | | |
|---|---|---|---|---|---|
| | | Sample 1 | Sample 2 | Sample 3 | Mean |
| | | | Embryos only | | |
| *days* | *gm.* | % | % | % | % |
| 12 | 1.4 | 1.88 | 1.81 | 1.84 | 1.84 |
| 15 | 6.3 | 2.06 | 2.20 | | 2.13 |
| 18 | 10.4 | 2.14 | 2.28 | 2.20 | 2.21 |
| 21 | 16.7 | 2.37 | 2.28 | 2.28 | 2.31 |
| | | | Yolks only | | |
| 12 | 20.6 | 1.00 | 1.00 | | 1.00 |
| 15 | 17.0 | 1.00 | 1.08 | | 1.04 |
| 18 | 11.5 | 0.84 | 0.84 | | 0.84 |
| 21 | 4.8 | 0.92 | 0.90 | | 0.91 |

points at about 9 days, suggesting the possibility of a downward curve of glycine destruction at the beginning of incubation, encountering in normal embryos an upward curve of glycine synthesis, which becomes pronounced at 12 days—approximately the age of highest mortality from chondrodystrophy.

If glycine in embryos and corresponding yolks is determined separately (table 2), and if the relative weight changes are

TABLE 3

*Glycine content of chondrodystrophic embryos without yolks*

| BREED | AGE AT DEATH | EMBRYOS | GLYCINE | | | |
|---|---|---|---|---|---|---|
| | | | Sample 1 | Sample 2 | Sample 3 | Mean |
| | *days* | *no.* | % | % | % | % |
| B.P.R.[1] | 10–14 | 10 | 1.40 | 1.56 | 1.40 | 1.45 |
| B.P.R. | 10–14 | 5 | 1.24 | 1.24 | 1.32 | 1.27 |
| B.P.R. | 14 | 3 | 1.16 | 1.00 | 1.00 | 1.05 |
| B.P.R. | 15 | 3 | 1.40 | 1.24 | 1.24 | 1.29 |
| B.P.R. | 20 | 1 | 1.40 | 1.24 | 1.40 | 1.35 |
| B.P.R. | 21 | 1 | 1.56 | 1.48 | 1.40 | 1.48 |
| B.P.R. | 15 | 3 | | | | 0.88 |
| B.P.R. | 19 | 1 | | | | 0.76 |
| B.P.R. | 19 | 1 | | | | 1.15 |
| Wh.L.[2] | 10 | 21 | | | | 0.78 |
| Wh.L. | 11 | 12 | | | | 0.88 |
| Wh.L. | 12 | 9 | | | | 1.00 |
| Wh.L. | 18 | 2 | | | | 1.57 |
| Wh.L. | 18 | 1 | | | | 0.95 |
| Wh.L. | 21 | 1 | | | | 1.64 |

[1] Barred Plymouth Rock.

[2] White Leghorn.

taken into account, the glycine synthesis is even more evident than in the whole egg.

The percentages of glycine found in chondrodystrophic embryos (table 3) fall far below those for normal embryos of comparable ages (tables 1 and 2).

It seems evident from these data that glycine is synthesized during development, and that chondrodystrophic embryos contain less than normal amounts of glycine. The development of cartilage, bone and down is greatly retarded in

chondrodystrophy. These tissues contain proteins high in glycine content. Whether the lack of glycine in chondrodystrophic chicks is a cause, or a result, of chondrodystrophy cannot be established by the present technique.

## SUMMARY

1. Glycine is synthesized during the development of White Leghorn and Barred Plymouth Rock chick embryos.

2. Chicks which die of chondrodystrophy during development contain less than normal amounts of glycine.

3. The nature of the relation between chondrodystrophy and the low value for glycine has not been established.

## LITERATURE CITED

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# IRON RETENTION IN INFANCY [1]

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FOUR FIGURES

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The iron content of the body at birth as determined by direct analysis (Camerer and Söldner, '00) averages about 350 mg. On the basis of existing data for tissue weights and iron content of those tissues richest in iron (Scammon, '23; Thoenes and Aschaffenburg, '34; White House Conference Reports, '32) it appears probable that the iron content of the body at 6 months may be no larger than at birth, while during the second half-year, it increases about 200 mg. It has been estimated that, during the first 2 months of life when hemoglobin normally is being destroyed, the average artificially fed infant excretes approximately 75 mg. more iron than he ingests (Stearns and McKinley, '37). To provide the iron necessary during the remainder of the first year of life, the infant would need to retain 0.75 to 1.0 mg. of iron daily after 2 months of age. The purpose of this study has been to determine the quantities of iron actually retained by infants from 7 to 54 weeks of age who are given various types of feeding.

Since the bulk of the infant's diet consists of milk, which is poor in iron, it has become customary to add to the diet some readily digestible food which is relatively rich in iron. Egg yolk is perhaps the most commonly used of these additional foods; cereals, vegetable and fruit purees are also given.

[1] This study was aided by a grant from Mead Johnson and Company, Evansville, Indiana.

Egg yolk, pureed spinach, and a special iron-rich cereal[2] were used for food sources of iron in this study.

The iron content of milk has been variously estimated as from 0.014 to 0.24 mg. per 100 cc. (table 1). A part of this variation is undoubtedly due to methods of analysis, a part to methods of handling the milk. These factors are probably greater than the differences among individual samples of milk.

The iron content of the additional foods used is also variable (Wallgren, '31–'32; Peterson and Elvehjem, '28). In this laboratory, analyses of egg yolks showed an average of 4.4 mg. of iron per 100 gm. Since yolks vary in weight from 12 to 22 gm., the addition of an egg yolk to the day's diet may be expected to increase the iron intake from 0.5 to 1.0 mg. daily. The pureed spinach used contained 1.22 mg. of iron per 100 gm., the cereal, 30 mg. per 100 gm.

The effect of other minerals in the diet upon the quantity of iron retained is a factor of importance because of the high mineral intake of infants. von Wendt ('05) observed that the addition of calcium sulfate to the diet of an adult man increased his iron retention. Shelling and Josephs ('34) found that in rats, an increased calcium intake decreased the retention of iron. These latter results were confirmed by Kletzien ('35) who reported also that increased intakes of sodium or potassium resulted in increased retention of iron by rats. No direct experiments have been found concerning the effect of the intake of other salts upon the retention of iron by infants. It has often been observed, however, that, although the iron content of human milk is also low, much more iron is retained by infants fed human milk than by those fed cow's milk. In addition to its lower phosphorus content, human milk contains more potassium and less calcium than cow's milk; potassium salts are often added to infants foods, in order to render the salt relationships more comparable to those of human milk. The effect upon the iron retention of the addition of potassium carbonate or chloride was therefore studied.

[2] Pablum.

TABLE 1

*Iron content of cow's milk*

| AUTHOR | DATE | METHOD OF HANDLING MILK | METHOD USED | IRON |
|---|---|---|---|---|
| | | | | *mg. per 100 cc.* |
| Sherman | 1907[1] | Commercial samples | | 0.24 |
| Edelstein-Csonka | 1911 | Drawn directly into glass containers | | 0.05 |
| Peterson-Elvehjem | 1928 | | Ferric thiocyanate | 0.24 |
| Telfer | 1930 | | | Range: 0.07–0.11<br>Average 0.08 |
| Cunningham | 1931 | Drawn directly into glass containers | | 0.065 |
| Davies | 1931 | Fresh Milk<br>Pasteurized<br>Sterilized | Ferric thiocyanate | Range:<br>0.15–0.24<br>0.17–0.38<br>0.16–0.28 |
| Stugart | 1931 | Certified commercial samples | Modification of ferric thiocyanate method | 0.044<br>0.046<br>0.053<br>0.073 |
| Reis and Chakmakjian | 1932 | Drawn directly into glass containers | Prussian blue | 0.14–0.15 |
| Wallgren | 1932 | Drawn directly into glass containers | Iodimetric titration | Range: 0.014–0.032<br>Average 0.024 |
| Rowett Research Institute[2] | | | | Range: 0.07–0.11<br>Average 0.08 |
| This laboratory | 1935 | Commercial samples | Stugart's modification of ferric thiocyanate method | 0.072<br>0.060<br>0.038 |

[1] Published in U. S. Dept. Agric. Bull., vol. 185, 1907; same value used in Sherman, Chemistry of Food and Nutrition, 1932.

[2] Quoted from Davidson and Leitch, Nutr. Abstr. and Rev., vol. 3, 1934.

Fourteen healthy infants served as subjects. Their hemoglobin values were determined at intervals during the study and averaged 11.9 gm. per 100 cc. One infant was given human milk; the basic milk formula for the others consisted of evaporated milk containing a cod liver oil concentrate. The milk feeding was acidified with citric acid, and contained 6% of added carbohydrate, a dry dextrin-maltose mixture. Distilled water was used for making up the feedings. Each infant was given 2 ounces of orange juice daily. The egg yolk, potassium and iron salts, when fed, were added to the formula, spinach and cereal were fed separately. Potassium chloride was given in amounts of 1.8 gm., and potassium carbonate, 1.67 gm. daily, approximately doubling the day's intake of potassium. From 1.25 to 5 cc. of a 1% solution of ferric ammonium citrate was added to the day's feeding when the retention of iron from a soluble iron salt was studied. The oldest infant (D.L.) after the age of 48 weeks, received other vegetables and fruits as well as those mentioned above. During the experimental periods, the day's feeding for each infant was quantitatively prepared and an aliquot saved for analysis. All refusals were carefully measured and subtracted from the daily intake. The infant was given the diet to be studied for several days prior to the experimental period, which was always of 3 days duration. Many short periods of study, rather than a few longer periods, were chosen because the necessary restriction of motion of the infant during the experimental periods is tiring, and it was felt that the error introduced by excessive fatigue of the infant would be greater than the analytical error introduced by the shortness of the period. The latter error is minimized by the number of periods studied. The copper intake was not determined. As none of the dietary constituents was especially purified, the copper content was assumed to be ample.

Diets and feces were digested with 20% iron-free HCl. Aliquot portions of these digests and of urine were ashed in a muffle furnace at just below red heat, the ash dissolved

in iron-free HCl and the iron determined according to the method of Stugart ('31). At the beginning of the study, porcelain crucibles were used for the ashing process, but it was observed that, as the glaze became attacked, a small iron blank was obtained from the crucible, particularly in those which had been used for ashing urine. Whenever sufficient material was available, the analyses were repeated, using platinum crucibles. The differences observed were very small, and did not affect materially the quantity of retention obtained; therefore all of the results are reported, even though for the studies of infants C.P., R.S. and E.T., porcelain crucibles were used in ashing some of the samples.

The results of the experiments are listed for each baby in table 2, and summarized graphically according to age in figure 1, and according to iron intake in figure 2.

When the milk formula was the sole source of iron, the day's intake varied from 0.66 to 2.06 mg., averaging 1.14 mg., or 0.19 mg. per kilogram. The range of retention was wide, from – 0.80 to 0.74 mg. a day, with an average daily loss of 0.05 mg., or slightly less than 0.01 mg. per kilogram. With this diet no consistent relationship could be observed between the intake level and the amount of iron retained (fig. 2) nor did the retention seem to vary in relation to the age of the infant (fig. 1).

When the diet was supplemented with egg yolk, the average intake was increased to 1.83 mg. daily (0.28 mg. per kilogram); the average retention was not increased. Pureed spinach was then given as a source of additional iron. Considerable difficulty was encountered in feeding the spinach and the average intake of iron was increased only to 1.25 mg. daily or 0.19 mg. per kilogram. The average iron loss was greater, – 0.11 mg. daily, than when the milk formula alone was given. Schlutz, Morse and Oldham ('33) fed infants dried, pureed and raw spinach and were able to increase the iron intake from 60 to 170%, but observed no significant increase in iron retention.

TABLE 2

*Intake and retention of iron by normal infants given various sources of dietary iron*

| INFANT | AGE | WEIGHT | DIET [1] | INTAKE PER KILOGRAM | RETENTION PER KILOGRAM | INFANT | AGE | WEIGHT | DIET [1] | INTAKE PER KILOGRAM | RETENTION PER KILOGRAM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *weeks* | *kg.* | | *mg.* | *mg.* | | *weeks* | *kg.* | | *mg.* | *mg.* |
| R.F. | 7 | 5.3 | B | 0.17 | + 0.02 | E.H. | 16 | 5.1 | B | 0.23 | + 0.03 |
| | 8 | 5.5 | B | 0.20 | 0.00 | | 18 | 5.5 | B | 0.29 | + 0.13 |
| | 12 | 6.1 | B | 0.22 | + 0.08 | | 22 | 5.9 | BS | 0.19 | — 0.04 |
| | 15 | 6.3 | BFe | 0.57 | + 0.04 | | 24 | 6.4 | BE | 0.25 | + 0.07 |
| | 17 | 6.9 | BFe | 0.58 | — 0.17 | | 25 | 6.5 | BFe | 0.42 | + 0.02 |
| | 18 | 7.1 | BFeK | 0.55 | + 0.25 | | 26 | 6.5 | B | 0.15 | + 0.05 |
| | 20 | 7.3 | BFeK | 0.32 | — 0.20 | | 27 | 6.7 | BES | 0.30 | + 0.07 |
| | 21 | 7.3 | BE | 0.27 | + 0.01 | | | | | | |
| | 23 | 7.7 | BE | 0.24 | + 0.04 | R.B. | 17 | 5.9 | B | 0.25 | — 0.14 |
| | 24 | 7.6 | BS | 0.12 | — 0.05 | | 18 | 6.1 | BS | 0.35 | + 0.08 |
| | 25 | 7.7 | BFe | 0.42 | + 0.11 | | 20 | 6.0 | B | 0.17 | — 0.06 |
| | 28 | 7.8 | BS | 0.17 | — 0.05 | | 24 | 6.8 | BE | 0.25 | + 0.01 |
| | 30 | 8.1 | BE | 0.31 | — 0.06 | | 25 | 6.9 | BS | 0.18 | — 0.01 |
| | 36 | 8.7 | BC | 0.56 | + 0.15 | | 26 | 6.5 | BS | 0.23 | + 0.03 |
| | | | | | | | 27 | 7.0 | BE | 0.26 | + 0.01 |
| R.Sc | 8 | 4.2 | B | 0.17 | — 0.03 | | | | | | |
| | 10 | 4.6 | B | 0.18 | — 0.01 | C.P. | 18 | 6.3 | BFe | 1.54 | + 0.07 |
| | 12 | 4.8 | B | 0.18 | — 0.10 | | 19 | 6.5 | BFe | 1.17 | + 0.51 |
| | 14 | 5.0 | BK | 0.14 | — 0.04 | | 20 | 6.8 | BFe | 1.38 | + 0.20 |
| | 16 | 5.1 | B | 0.20 | + 0.01 | | 22 | 7.7 | BFeK | 1.30 | — 0.66 |
| | 17 | 5.2 | BFe | 4.20 | + 0.88 | | 23 | 7.9 | BFeK | 1.38 | + 0.39 |
| | 21 | 5.3 | BFe | 1.1 | + 0.23 | | 26 | 8.6 | BFeK | 0.94 | — 0.63 |
| | 22 | 5.4 | BKFe | 0.79 | + 0.10 | | | | | | |
| | 23 | 5.7 | BKFe | 1.25 | + 0.43 | D.L. | 18 | 5.6 | B | 0.18 | — 0.04 |
| | 27 | 6.1 | BE | 0.45 | — 0.06 | | 20 | 5.9 | B | 0.21 | — 0.04 |
| | 28 | 6.2 | BE | 0.45 | — 0.02 | | 21 | 5.9 | BS | 0.17 | — 0.06 |
| | 34 | 6.3 | BE | 0.26 | — 0.04 | | 22 | 6.1 | BE | 0.19 | — 0.03 |
| D.G. | 4 | 5.3 | B | 0.26 | + 0.10 | | 26 | 6.1 | BFe | 0.66 | + 0.36 |
| | 9 | 5.7 | B | 0.16 | — 0.01 | | 27 | 6.2 | B | 0.14 | + 0.01 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 23 | 5.7 | BKFe | 1.25 | + 0.43 | D.L. | 18 | 5.6 | B | 0.18 | — 0.04 |
| | 27 | 6.1 | BE | 0.45 | — 0.06 | | 20 | 5.9 | B | 0.21 | — 0.04 |
| | 28 | 6.2 | BE | 0.45 | — 0.02 | | 21 | 5.9 | BS | 0.17 | — 0.06 |
| | 30 | 6.3 | BE | 0.53 | — 0.03 | | 25 | 6.1 | BFe | 0.19 | — 0.02 |
| D.G. | 7 | 5.5 | B | 0.26 | + 0.10 | | 26 | 6.1 | BFe | 0.55 | + 0.36 |
| | 9 | 5.7 | B | 0.16 | — 0.01 | | 27 | 6.2 | B | 0.14 | + 0.01 |
| | 11 | 6.1 | BFe | 0.50 | + 0.04 | | 29 | 6.9 | BES | 0.40 | 0.0 |
| | 12 | 6.3 | BE | 0.26 | — 0.08 | | 36 | 7.7 | BEC | 0.47 | + 0.06 |
| | 13 | 6.4 | BS | 0.12 | — 0.04 | | 37 | 7.8 | BEC | 0.49 | + 0.17 |
| | 15 | 6.8 | BE | 0.22 | — 0.04 | | 48 | 8.5 | BE | 0.32 | + 0.11 |
| | 16 | 6.8 | BES | 0.25 | — 0.01 | | 49 | 8.4 | BE | 0.32 | + 0.09 |
| | | | | | | | 53 | 8.4 | BEC | 0.81 | + 0.16 |
| M.M. | 10 | 5.0 | B | 0.32 | + 0.04 | | 54 | 8.6 | BEC | 0.79 | + 0.09 |
| | 12 | 5.4 | B | 0.12 | — 0.04 | | | | | | |
| | 14 | 5.7 | BFe | 0.91 | + 0.32 | R.Sw. | 23 | 6.6 | BFe | 1.36 | + 0.68 |
| | 16 | 6.1 | BS | 0.20 | — 0.01 | | 24 | 6.6 | BFe | 1.50 | + 0.28 |
| | 17 | 6.2 | BE | 0.25 | — 0.07 | | 27 | 7.2 | BFeK | 1.34 | + 0.16 |
| | 19 | 6.4 | BE | 0.24 | — 0.01 | | 28 | 7.2 | BFeK | 1.34 | + 0.20 |
| | 21 | 6.9 | BE | 0.18 | + 0.12 | | 31 | 7.6 | BFeK | 1.38 | + 0.03 |
| C.B. | 11 | 5.8 | B | 0.18 | — 0.01 | E.T. | 25 | 6.6 | B | 0.21 | — 0.02 |
| | 13 | 6.1 | B | 0.16 | — 0.01 | | 26 | 6.9 | B | 0.19 | — 0.04 |
| | 15 | 6.4 | B | 0.11 | — 0.04 | | 28 | 7.3 | B | 0.20 | + 0.01 |
| | 16 | 6.5 | BFe | 0.40 | — 0.02 | | 29 | 7.3 | B | 0.16 | + 0.03 |
| | 21 | 7.2 | B | 0.11 | — 0.07 | | 31 | 7.7 | BK | 0.21 | — 0.04 |
| | 28 | 8.5 | BC | 0.40 | + 0.09 | | 32 | 7.8 | BK | 0.26 | + 0.01 |
| R.M. | 12 | 5.8 | B | 0.24 | — 0.06 | G.S. | 9 | 5.8 | HM | 0.06 | 0.0 |
| | 13 | 6.2 | BFe | 0.29 | — 0.07 | | 13 | 5.7 | HM | 0.08 | 0.0 |
| | 15 | 6.3 | BFe | 0.42 | — 0.12 | | 15 | 6.9 | HM | 0.08 | + 0.03 |
| | 25 | 7.6 | BFe | 0.58 | + 0.12 | | 18 | 7.2 | HME | 0.17 | + 0.06 |
| | | | | | | | 21 | 7.4 | HME | 0.15 | + 0.06 |
| R.N. | 13 | 6.5 | B | 0.20 | 0.0 | | 23 | 7.8 | HME | 0.15 | + 0.02 |
| | 14 | 6.6 | BFe | 0.43 | — 0.22 | | 24 | 8.0 | HME | 0.14 | + 0.04 |
| | 16 | 6.9 | BFe | 0.39 | — 0.46 | | 25 | 8.2 | HMBE | 0.12 | +0.05 |
| | 28 | 8.4 | BFe | 0.61 | + 0.01 | | 26 | 8.5 | BE | 0.25 | + 0.02 |

[1] B = basal diet; K = potassium chloride or carbonate; Fe = ferric ammonium citrate; E = egg; S = spinach; C = special cereal mixture; HM = human milk.

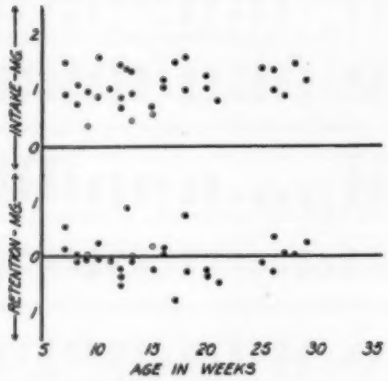


Fig. 1 Daily intakes and retentions of iron of infants from 5 to 30 weeks of age. The dots represent studies of infants given evaporated milk feedings; the circles, studies of an infant given human milk.

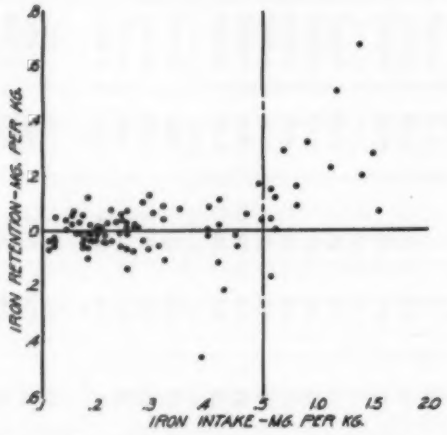


Fig. 2 The relation of iron retention to intake. Data plotted as milligrams of iron per kilogram of body weight daily.

The quantity of spinach given to the infants of the present study, while insufficient to increase the iron intake materially, did affect the retention of calcium by the infants. During the thirteen periods when spinach was given, the calcium retention averaged 27% of the intake whereas during the twenty-six periods of study preceding and following the spinach feeding, these same infants retained 35% of the calcium intake. It is admitted that the spinach feeding was not continued for more than 10 days at a time, so the findings are not representative of a diet to which the baby has become fully adjusted. It is doubtful whether spinach would be a constant part of any infant's diet, so probably few infants would handle it better than these. In general, therefore, the feeding of spinach to infants seems more detrimental than beneficial, at least in so far as calcium and iron are concerned.

During three periods of study, both egg yolk and spinach were added to the diet. The intake of iron averaged 2.14 mg. daily or 0.32 mg. per kilogram, the average daily retention was 0.13 mg. or 0.02 mg. per kilogram, a positive though very small retention.

In view of the failure of these food sources of iron to increase sufficiently the amounts retained by the infants, the question arose as to whether infants of these ages could retain iron added to the diet in any form. A solution of ferric ammonium citrate was then added to the feedings in amounts sufficient to give daily iron intakes of from 2.6 to 21.8 mg., or from 0.29 to 4.20 mg. per kilogram. Of the seven periods of study wherein the iron intake was below 0.5 mg. per kilogram, only two showed a positive retention. The average intake of this group was 0.39 mg. per kilogram daily, the average retention −0.17 mg. per kilogram, a poorer retention than that observed from the diets alone. When the iron intake was increased to from 0.5 to 1.0 mg. per kilogram daily, only one negative balance was found, the average retention for the six periods of study being 0.09 mg. per kilogram, with an average intake of 0.64 mg. per kilogram. A further increase in intake to from 1.0 to 1.6 mg. per kilogram

resulted in very good retentions. The intake averaged 1.34 mg. per kilogram, for the six periods studied, and the retention averaged 0.33 mg. per kilogram, or approximately 24% of the intake. It thus appears that the chief factor in determining the quantity of iron retained is the quantity ingested and that intakes of iron below 0.5 mg. per kilogram a day are insufficient to permit constant positive retentions by the average infant fed cow's milk.

To test this theory with iron of the diet from food sources, a special cereal mixture unusually rich in food iron was given in amounts of from 7 to 15 gm. daily. Egg yolk was also added to some of the diets. The daily iron intakes were adjusted to approximate 0.5 mg. per kilogram, and varied from 0.40 to 0.81 mg. per kilogram, or from 3.41 to 6.81 mg. for the total daily ingestion. Positive retentions of iron were obtained in all six periods of study, averaging 20% of the intake. When the intake was between 0.4 and 0.5 mg. per kilogram, the average retention was 0.10 mg. per kilogram, in contrast to the negative balance of – 0.17 mg. per kilogram observed when ferric citrate was the chief source of added iron. With intakes above 0.5 mg. per kilogram, averaging 0.72 mg. per kilogram, the retentions varied from 0.09 to 0.16 mg. per kilogram with an average retention of 0.13 mg. per kilogram. It seems from these experiments that iron from food sources, when given in sufficient amounts, is at least as well handled as is iron fed as ferric salt.

In figure 2, the intakes of iron per kilogram body weight have been plotted against the retention per kilogram. The data include the periods of study with the basal diet, and the basal diet plus egg, spinach, cereal, or iron salts. The chart indicates that, regardless of the source of iron in the diet of the artificially fed infants, the average retention is negligible when the daily intake is less than 0.5 mg. per kilogram. The failure of egg or spinach feeding in increasing the iron retention can be explained wholly by the fact that neither increased the iron intake sufficiently. From the retentions observed in these studies, an intake of from 1.0 to 1.5 mg.

of iron per kilogram body weight would seem ample to permit sufficient retentions of iron. These amounts are much lower than the intake advised by Elvehjem and his co-workers ('35). These authors found that maximum increases in hemoglobin level were obtained when 25 mg. of iron and 1 mg. of copper were given daily, and that increases in hemoglobin were not so consistently observed when the intakes were below this level.

### RETENTION OF IRON BY AN INFANT FED HUMAN MILK

One infant (G.S.) was given human milk for three periods of study, then human milk supplemented with egg yolk, with egg yolk and cow's milk, and finally was given the same basal diet as the other infants studied, together with an egg yolk daily. The daily intake of iron increased from 0.45 mg. during the first period to 1.14 mg., when supplemented with egg yolk, and to 2.11 mg. for the cow's milk-egg yolk diet. The baby neither lost nor retained iron during the first two periods, but retained some iron during each succeeding period studied. The iron retained when human milk only was fed, averaged 0.08 mg. daily or slightly more than 0.01 mg. per kilogram The addition of egg yolk increased the daily retention to 0.32 mg. (0.045 mg. per kilogram). When the milk of the diet was supplied by equal portions of cow's and human milk, given as separate feedings, the iron intake was approximately the same, 1.03 mg., and the daily retention 0.39 mg., a retention quite comparable to that when human milk and egg alone were fed. During the one period when no human milk was given, although the daily intake of iron was doubled, 2.11 mg., the daily retention dropped to 0.20 mg. Wallgren ('33) found that three of five infants given human milk retained satisfactory quantities of iron. Other investigators (Krasnogorsky, '06; Telfer, '30) have observed, in general, much greater retention of iron from human than from cow's milk, and Maurer and his collaborators ('34) observed that substitutions of human milk for a part of the cow's milk of the feeding increased the iron retention of the infant.

THE EFFECT OF INTAKE OF PHOSPHORUS, CALCIUM AND POTASSIUM UPON IRON RETENTION

It seemed logical that the high phosphate content of cow's milk, relative to its iron content, might be the factor responsible for the poorer absorption of iron from cow's than from human milk. The iron retention per kilogram daily was therefore plotted against the intake ratio of phosphorus to iron (fig. 3). The dots represent iron intakes of 0.5 mg. per kilogram or above; the circles, iron intakes below 0.5 mg. per kilogram and the triangles, the periods when human milk

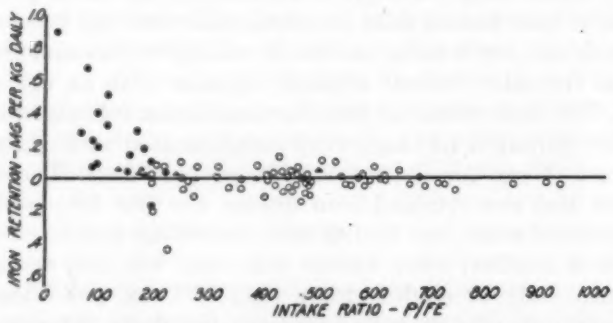


Fig. 3 The relationship retention of iron and the phosphorus: iron intake ratio. Dots represent intakes of iron greater than 0.5 mg. per kilogram daily; the circles, iron intakes less than 0.5 mg. per kilogram daily; the triangles, iron retention from human milk.

was fed. The values obtained indicate that the phosphorus intake could be increased from 200 to 1000 times the iron intake without effect upon the iron retention. The increasing iron retentions as the phosphorus: iron intake ratio decreased below 200, can be ascribed solely to the increase in iron intake. The iron retentions from human milk are no greater than many of the retentions observed when the babies were fed cow's milk.

The iron retentions per kilogram daily compared with the calcium: iron intake ratios (fig. 4), show a curve similar to that obtained with the phosphorus: iron values. Consistent

increases in iron retention were always observed when the iron intake was increased to more than 0.5 mg. per kilogram, but increasing the calcium intake from 200 to 1200 times the iron intake was without effect upon the iron retention, regardless of whether the source of iron and calcium was human or cow's milk. These findings accord with neither those of von Wendt ('05) nor Kletzien ('35).

To study the possible effect of the potassium intake upon the iron retention, potassium chloride or carbonate was added to the feeding in amounts sufficient to double the potassium

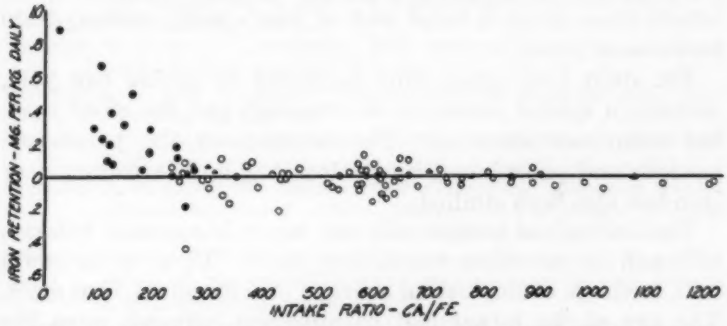


Fig. 4 Relationship between retention of iron and calcium: iron intake ratios. Symbols as in figure 3.

intake. Ferric ammonium citrate was also added. The average intake of iron was 0.86 mg. per kilogram, the average retention 0.00. This very poor average retention was due chiefly to two periods of study of baby C.P. When potassium salts were first added to his diet the result was a heavy loss of iron from the body; after the salt had been in the diet for about 2 weeks, the baby again retained iron. After a week without the salt addition, when KCl was replaced in the diet, the retention again became strongly negative. Omitting the periods of study of this baby, the average intake of iron was 0.76 mg. per kilogram, the retention 0.09 mg. per kilogram or 12%. Of eleven control periods studied, using the same infants given iron but not potassium salts, the average intake

was 0.91 mg. per kilogram, the average retention 0.18 mg. per kilogram or 20%. The age range of the infants was the same. These results do not confirm those of Kletzien ('35) upon rats, and indicate that the addition of potassium salts to the infant dietary is of no benefit to and may have a deleterious effect upon the iron retention.

## SUMMARY

The retention of iron by fourteen infants varying from 7 to 54 weeks of age has been studied throughout a total of 107 3-day balance periods. One infant was fed human milk, the others were given a basal diet of cow's milk, carbohydrate and orange juice.

The daily iron intake was increased by giving egg yolk, spinach, a special cereal, or an iron salt, and the effect upon the retentions observed. The influence of the potassium, calcium and phosphorus intake levels upon the retention of iron has also been studied.

The baby given human milk was never in negative balance, although the retention was always small. Those given cow's milk feedings alone, lost an average of 0.05 mg. of iron daily. The age of the infant had no apparent influence upon the ability to retain iron. Neither egg yolk nor spinach, in the amounts given, increased the iron retention. The retention was definitely increased when the infants were given the special iron-rich cereal or ferric ammonium citrate.

No consistent relationship was observed between the iron retention and the intake of potassium, calcium or phosphorus.

From studies of the iron retention after the ingestion of varying amounts of iron as food or as soluble salt, it appears that an intake of approximately 0.5 mg. per kilogram body weight is necessary to insure a retention of iron, and an intake of 1 to 1.5 mg. per kilogram permits ample retention. Ample retentions were observed with these intakes of iron, whether the source of iron was from food (special cereal and egg) or from a ferric salt (ferric ammonium citrate).

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# THE CONSERVATION OF BLOOD IRON DURING THE PERIOD OF PHYSIOLOGICAL HEMOGLOBIN DESTRUCTION IN EARLY INFANCY [1]

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ONE FIGURE

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The amount of hemoglobin in the blood at birth is higher than at any time subsequently and averages 22 to 23 gm. per 100 cc.[2] A rapid decrease occurs during the first weeks of life. Wide individual differences are observed in the duration of the period of hemoglobin destruction, but by the tenth week of life the amount of hemoglobin has usually decreased to 11 to 13 gm. per 100 cc., at which value it remains with slight fluctuations during the next 2 years. The purpose of this report is to record the results of an investigation concerning the fate of the iron freed by the breakdown of hemoglobin during the early weeks of life.

Studies of many years ago showed that the human fetus accumulates some store of iron in the liver and spleen. Because of the small size of the spleen, its total storage is almost negligible. The liver weighs approximately 135 gm. at birth (Scammon, '23) and, according to some observations, contains 0.026% of non-hemoglobin iron (Thoenes and Aschaffenburg, '34) or a total of 35 mg. Other data indicate an average total storage of 40 to 60 mg. (Gladstone, '32; Toverud, '35).

[1] This study was aided by a grant from Mead Johnson and Company, Evansville, Indiana.

[2] Throughout this discussion, whenever several sources are quoted, references are omitted. The major articles are listed in the reference list.

Strauss ('33) has shown recently that the liver storage is inconstant and may be negligible if the diet of the mother has been low in iron. Thus the amount of iron in the liver at birth may vary from a negligible quantity to a total of possibly 60 mg. At 6 months of age the average weight of the liver is 240 gm. and its stored iron has decreased to 0.006%, or a total of 15 mg. (Scammon, '23; Thoenes and Aschaffenburg, '34). During the first 6 months of life the blood volume increases 200 cc. or more. On the basis of the average value of 12 gm. of hemoglobin per 100 cc. of blood and the content of 3.35 mg. of iron in each gram of hemoglobin (Butterfield, '09), the 200 cc. increase in blood represents 80 mg. of iron. This amount alone, exclusive of the iron needed for muscle growth, is considerably greater than the difference between the amount of iron stored at birth and that present at 6 months of age, or greater even than the total storage at birth. Obviously the iron stored in the liver at birth cannot represent the major source of iron for the formation of new blood and tissue.

The postnatal decrease of 10 to 12 gm. of hemoglobin per 100 cc. of blood in a baby whose total blood volume approximates 450 cc., liberates 150 to 180 mg. of iron, an amount sufficient for the formation of 300 to 450 cc. of new blood if the iron can all be stored for subsequent utilization. Thus the amount of iron freed by the physiological destruction of hemoglobin in early infancy exceeds greatly the amount in storage at the time of birth and obviously is the infant's largest potential source of iron. It is therefore desirable to ascertain what proportion of the freed iron is salvaged by the infant.

The evidence in the literature concerning iron conservation in early infancy is incomplete. Gladstone ('32) made both chemical and histological studies of the iron content of human fetuses and infants from the fourth month of intrauterine to the third month of postnatal life and found the maximum iron content of the liver was reached after birth, at from 1 to 10 weeks of age. This finding constitutes evidence that some storage occurred of the iron released during the period of

physiological blood destruction. Evidence that not all the released iron is stored is found in the studies of Beck ('32) who observed that iron excretion increased from the second day after birth and reached a maximum between the thirtieth and the fortieth day, then gradually decreased. Other investigators have found fecal iron values of young infants fed human milk varying from 0.35 to 2.57 mg. daily. Langstein and Edelstein ('13) studied a breast-fed newborn infant and found in consecutive 5-day studies, losses of 0.65, 1.28, 0.04 and 0.12 mg. daily, whereas two infants, 8 and 12 weeks of age, retained 0.18 and 0.26 mg. daily, respectively. On the other hand, Lichtenstein ('21) studied four breast-fed infants from 3 to 19 weeks of age and found no positive retentions in any of the twelve periods of study. The losses varied from 0.02 to 0.48 mg. daily, with an average loss of 0.25 mg. per day. The quantity of loss bore no consistent relation to age. Stearns and Stinger ('37) found that infants from 7 to 29 weeks of age, given feedings of cow's milk mixtures, showed variable retentions and losses but averaged the slight loss of 0.05 mg. daily. Again no relation was observed between age and iron retention or loss.

The evidence at hand thus indicates that during the early weeks of life the excretion of iron almost invariably exceeds the intake, but that after the second month the iron balance, though variable, tends to approach zero.

As the total amount of iron which disappears from the blood varies with the individual infant, it is necessary to follow both the level of blood iron and the daily excretion and intake in order to determine the quantity of iron freed from hemoglobin but not excreted from the body. In this study determinations of blood iron were carried out at short intervals and iron excretion was observed for several periods of varying length each during the first 2 months of life.

The infants were brought under observation as soon after birth as possible, usually at 10 days of age. The excreta were collected on pads of cellucotton. The collection for the entire period was digested with 10% iron-free HCl until a homogeneous suspension resulted. The mixture was cooled, diluted

to a convenient volume, thoroughly mixed and aliquots taken for analysis. Iron was determined by Stugart's method ('31), correcting for the iron content of the cellucotton and reagents used. Urinalyses were not made, as the iron content of urine of young infants is negligible. The iron content of each of the separate foods used, evaporated milk, a dry dextrin-maltose mixture, orange juice, and distilled water, was determined and the daily iron intake of each infant calculated.

For the blood studies, venous blood was taken from the longitudinal sinus, oxalated and well mixed. Hemoglobin was determined by the Newcomer method ('23) using a standard calibrated color disc. Cell volume was obtained by the use of the Van Allen hematocrit ('25); standard pipettes and hemocytometers were employed in making cell counts. Fowweather's method ('26) for iron content of blood was followed except for the substitution of amyl alcohol for acetone in extracting the ferric thiocyanate from the dilute aqueous solution. Standard iron solutions for both blood and fecal iron determinations were prepared from pure iron wire.

The iron excretion of seven infants was studied during the period of blood destruction. As it was deemed undesirable to take blood samples too frequently from the same infant, a larger group was used for the blood study. An average of seven analyses for each week of age was obtained from the seventeen infants studied in this series.

## BLOOD VALUES

Table 1 contains a summary of the data from determinations of the cell volume, number of erythrocytes per cubic millimeter, hemoglobin and iron content of the blood of these infants. The individual values for each determination are shown graphically in figure 1, the average values at birth being taken from the literature, and the average curves from the values of table 1.

It will be observed from the chart that, while each of the plotted components is at its highest level at birth and reaches

TABLE 1

*Summary of blood data*

| AGE | NUMBER OF INFANTS | AVERAGE WEIGHT | CELL VOLUME | RED BLOOD CELLS | HEMOGLOBIN | | | CALCULATED BLOOD IRON FROM HEMOGLOBIN[1] | DETERMINED BLOOD IRON | ESTIMATED TOTAL BLOOD IRON[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Minimum | Maximum | Average | | | |
| *days* | | *gm.* | *%* | *millions per cu. mm.* | *gm. per 100 cc.* | | | *mg. per 100 cc.* | *mg. per 100 cc.* | *mg.* |
| 10–14 | 5 | 3426 | 36.8 | 4.45 | 14.55 | 21.04 | 17.18 | 57.6 | 58.4 | 216 |
| 14–21 | 10 | 3590 | 34.9 | 4.44 | 12.40 | 20.14 | 15.58 | 52.2 | 51.7 | 194 |
| 21–28 | 10 | 3793 | 30.8 | 4.16 | 12.05 | 16.91 | 14.48 | 48.5 | 46.1 | 195 |
| 28–35 | 12 | 4040 | 27.0 | 3.92 | 10.51 | 15.08 | 12.41 | 41.6 | 39.2 | 168 |
| 35–42 | 8 | 4235 | 25.3 | 3.77 | 10.28 | 13.96 | 11.70 | 39.3 | 37.3 | 172 |
| 42–49 | 10 | 4465 | 25.9 | 3.91 | 9.23 | 15.08 | 11.82 | 39.6 | 39.2 | 189 |
| 49–56 | 7 | 4485 | 23.9 | 4.05 | 9.79 | 12.11 | 10.52 | 35.2 | 35.2 | 185 |
| 56–63 | 10 | 5051 | 25.9 | 3.49 | 10.20 | 12.05 | 10.82 | 36.3 | 34.0 | 184 |
| 63–70 | 6 | 4876 | 26.8 | 3.54 | 9.71 | 12.51 | 10.69 | 35.7 | 32.8 | 176 |
| 70–77 | 5 | 5614 | 26.1 | 3.40 | 10.52 | 11.48 | 11.15 | 37.3 | 33.2 | 224 |
| 77–84 | 8 | 5638 | 25.8 | 3.72 | 10.44 | 12.05 | 10.99 | 36.9 | 34.7 | 215 |
| 84–91 | 6 | 5583 | 25.4 | 3.52 | 10.11 | 11.71 | 11.11 | 37.2 | 33.8 | 211 |
| 91–98 | 8 | 5717 | 26.3 | 3.66 | 9.02 | 11.48 | 10.58 | 35.4 | 32.7 | 206 |
| 98–105 | 2 | 5588 | 25.0 | 4.06 | | | | | 33.3 | 204 |
| 105–112 | 5 | 5367 | 29.0 | 3.51 | 10.11 | 12.28 | 10.98 | 36.8 | 33.1 | 194 |
| 112–119 | 4 | 6165 | 29.1 | 3.74 | 11.14 | 11.38 | 11.27 | 37.7 | 34.4 | 232 |
| 119–126 | 6 | 6105 | 30.6 | 4.14 | 11.00 | 12.05 | 11.58 | 38.8 | 35.2 | 235 |
| 126–140 | 5 | 6031 | 28.6 | 4.25 | 11.05 | 12.17 | 11.52 | 38.6 | 34.3 | 226 |
| 140–194 | 8 | 6211 | 29.5 | 3.70 | 11.10 | 13.19 | 11.80 | 39.5 | 33.1 | 225 |

[1] The blood volume up to the fourteenth day was taken as 14.7% of body weight, and after the fourteenth day as 10.9% of body weight (Lucas and Dearing, '21). The Butterfield ('09) factor, 3.35 mg. of iron per gram of hemoglobin, was used in converting hemoglobin to iron.

a minimum at approximately the same time, less parallelism is observed between the cell volume, erythrocyte count and hemoglobin than perhaps might be expected. The hematopoietic mechanism of the infant is most unstable; the blood may be flooded with immature red cells, and its water content may vary from day to day (Abt, '35). In fact, Drucker ('24)

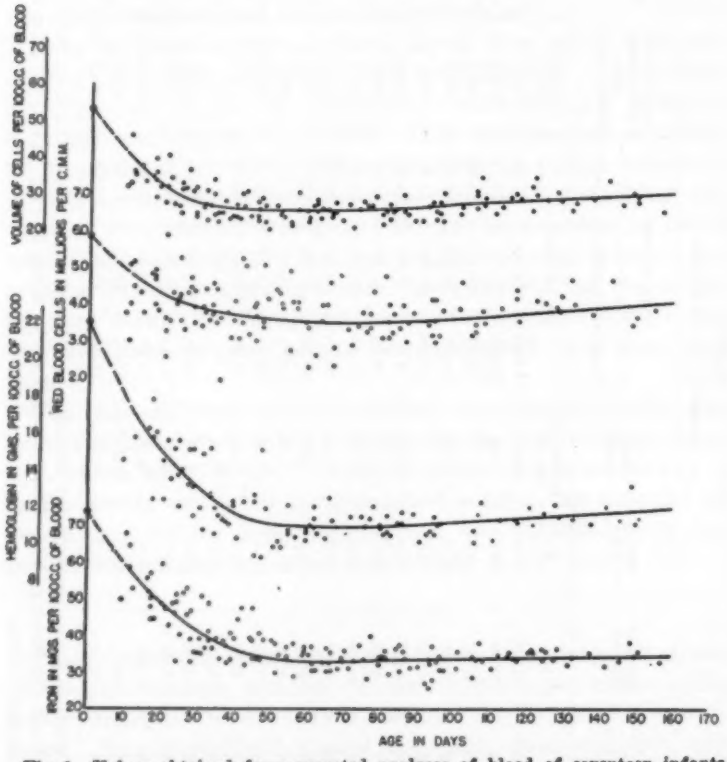
VOLUME OF CELLS PER 100CC. OF BLOOD
RED BLOOD CELLS IN MILLIONS PER C.MM.
HEMOGLOBIN IN GMS. PER 100CC. OF BLOOD
IRON IN MGS. PER 100CC. OF BLOOD
AGE IN DAYS

Fig. 1 Values obtained from repeated analyses of blood of seventeen infants between 10 and 160 days of age. The average values at birth are estimated; the curves are drawn from the average values obtained at the different ages. Cell volumes and hemoglobin are shown in per cent, red cells in millions per cubic centimeter of blood; and iron in milligrams per 100 cc. Sinus blood was used, except for a few hemoglobin determinations noted by +, which were determined on capillary blood.

has commented that the range of hemoglobin in the first 2 weeks of life is too great for the determination to have any clinical significance. Notwithstanding the variability, certain definite trends are observed.

The cell volume, which was estimated as 54% at birth (Mugrage and Andresen, '36), decreased to 24% by the seventh to the eighth week and then increased with minor fluctuations, to 30% at about the twentieth week. Mugrage and Andresen ('36) reported a minimal cell volume of 34% occurring between the eighth to the sixteenth week, with an increase to 37% by the thirty-second week.

The erythrocyte count at birth was estimated from the data in the literature as 5.85 millions per cubic millimeter. The values obtained are exceedingly variable during the first weeks of life and the number of erythrocytes is not as consistent with the cell volume as is observed in older infants and children. The average red cell count had decreased to 3.92 millions per cubic millimeter by the sixth week and the minimal value of 3.4 millions per cubic millimeter was reached by the eleventh week of age. The number of erythrocytes then increased to about 4 millions per cubic millimeter. Other average minimal values reported vary from 3.10 millions per cubic millimeter at 10 weeks (McLean and Caffey, '25) to 4.61 millions per cubic millimeter at 15 weeks (Merritt and Davidson, '33).

The hemoglobin at birth averages about 22 gm. per 100 cc. At the beginning of the study when the infants were 10 to 14 days of age, the average hemoglobin was 17.2 gm. per 100 cc., a value lower than the 22.2 gm. per 100 cc. reported by Elvehjem et al. ('33) and the 18.9 gm. reported by Appleton ('18). At 2 to 3 weeks the average hemoglobin was 15.6 gm., approximately equal to that observed by Mackay ('33) and higher than the average of 14.1 gm. observed by Appleton ('18). Other investigators, however, have found from 16 to 18 gm. of hemoglobin per 100 cc. in infants from 2 to 4 weeks of age. The lowest hemoglobin level, 10.5 gm. per 100 cc., was observed during the seventh and the eighth

week of life. The minimal hemoglobin level is usually reported to occur between the fifth and the twelfth week, the values as determined by different authors varying from 9.6 to 12.6 gm. per 100 cc.

The hemoglobin iron was calculated by using 3.35 mg. as the amount of iron per gram of hemoglobin (Butterfield, '09). Until about the fourteenth day of life the determined blood iron for each infant was greater than the calculated hemoglobin iron, the average values being 58.4 and 57.6 mg. per 100 cc., respectively. After this period of rapid fall the calculated hemoglobin iron was consistently greater than the determined iron. The minimal iron value of 32.8 mg. per 100 cc. was reached at 9 to 10 weeks of age, about 2 weeks later than the low level for hemoglobin. During the latter part of the study the values approached 35 mg. of iron per 100 cc. of blood.

In comparison with values of many other workers the hematologic values in this study tend to be low. The use of oxalated venous blood instead of capillary blood as is customary, could not have been the major cause. According to Lucas and Dearing ('21), hemoglobin is higher in sinus blood than in capillary blood during the first 2 weeks of life, while the number of erythrocytes in sinus blood is at first higher than and then equals that in capillary blood. A second possible factor is that with two exceptions the infants of this study were artificially fed. Other workers have studied chiefly infants fed human milk. It is well known that the iron of human milk is better retained by the infant than the same amount of iron in cow's milk. The blood values of the two infants of this study given human milk, were not conspicuously different from the values of the infants given cow's milk. The third and most obvious possible cause of the low hemoglobin values is the quantity of blood removed for study. As several factors were studied and the determinations were carried out in duplicate, 3 cc. samples of blood were taken. The total quantity of blood removed from an infant varied from 3 to 60 cc. After correction for the 20 to 25% of freed

iron which is excreted from the body, as will be discussed subsequently, the cumulative effect of several successive removals of these small amounts of blood is equivalent to the removal of sufficient iron to permit an average hemoglobin content at 20 weeks of age of from 0.3 to 1.0 gm. greater per 100 cc. of blood than the amount actually found. The removal of 3 cc. of blood weekly over a period of 6 to 20 weeks may thus be a real factor in depleting blood hemoglobin. Correcting for the average amounts of blood lost, the minimal hemoglobin values at 10 weeks would be 11.10 instead of 10.67 gm. per 100 cc. and the average value at 20 weeks, 12.27 instead of 11.80 gm. per 100 cc. These values approximate the averages found by others.

### IRON BALANCE STUDIES

Table 2 shows the average daily intake, fecal excretion and loss of iron of seven infants between the ages of 11 and 57 days. The twenty-six periods of study varied from 1 to 11 days in length, averaging 4 days each. The iron intake varied from 0.64 to 1.01 mg. a day with a group average of 0.76 mg. daily. The average daily excretion for any single period varied from 0.92 mg. to 4.33 mg., and while each baby showed considerable variation in the amount of iron excreted, certain infants, notably D.A. and D.Sh., consistently excreted more iron than others. D.L. had been born prematurely, weighing 2458 gm. at birth. His average daily iron excretion was practically identical with the average excretion of the group.

In no period studied was the intake greater than the excretion. The smallest loss, 0.25 mg. was observed during the last period of study of R.P. at 1 month of age. The greatest daily loss was 3.60 mg. and the average for the entire group was 1.22 mg. daily.

The relative daily iron loss of an infant bore no apparent relation to the length of the period of blood destruction. The duration of this period varied markedly among the several infants, the extremes being 21 and 76 days, respectively.

TABLE 3

*Daily iron balance of infants during period of hemoglobin destruction*

| NAME. AGE AT LOWEST BLOOD Fe | AGE | AVERAGE WEIGHT | AVERAGE DAILY INTAKE | AVERAGE DAILY FECAL EXCRETION | AVERAGE DAILY LOSS |
|---|---|---|---|---|---|
| | *days* | *gm.* | *mg.* | *mg.* | *mg.* |
| R.P. 31 days | 12–15 | 3495 | 0.71 | 1.63 | 0.92 |
| | 19–21 | 3700 | 0.62 | 1.62 | 1.00 |
| | 25–27 | 3930 | 0.69 | 1.35 | 0.66 |
| | 27–28 | 3960 | 0.71 | 3.35 | 2.64 |
| | 32–34 | 4297 | 0.69 | 0.94 | 0.25 |
| Average | | | 0.68 | 1.60 | 0.92 |
| R.D. 36 days | 11–14 | 3750 | 0.54 | 1.62 | 1.08 |
| | 17–19 | 3800 | 0.63 | 1.83 | 1.20 |
| | 23–25 | 3965 | 0.66 | 2.85 | 2.19 |
| | 29–30 | 4125 | 0.69 | 1.40 | 0.71 |
| | 37–39 | 4362 | 0.73 | 1.87 | 1.14 |
| Average | | | 0.64 | 1.94 | 1.30 |
| H.E. 21 days | 16–20 | 3975 | 0.70 | 1.14 | 0.44 |
| | 21–25 | 4155 | 0.81 | 1.97 | 1.16 |
| | 25–28 | 4317 | 0.62 | 1.32 | 0.70 |
| | 28–32 | 4425 | 0.63 | 2.82 | 2.19 |
| Average | | | 0.69 | 1.83 | 1.14 |
| D.Sh. 40 days | 20–28 | 3192 | 0.70 | 3.06 | 2.36 |
| | 28–30 | 3415 | 0.76 | 2.43 | 1.67 |
| | 30–31 | 3472 | 0.82 | 1.30 | 0.48 |
| | 31–38 | 3637 | 0.82 | 2.39 | 1.57 |
| Average | | | 0.76 | 2.63 | 1.87 |
| D.A. 29 days | 16–19 | 3167 | 0.62 | 4.22 | 3.60 |
| | 27–34 | 3642 | 1.07 | 2.19 | 1.12 |
| | 34–45 | 4027 | 1.07 | 2.36 | 1.29 |
| Average | | | 1.01 | 2.57 | 1.56 |
| D.Sm. 76 days | 25–32 | 4400 | 0.73 | 1.30 | 0.57 |
| D.L. (prematurely born) 69 days | 24–27 | 3005 | 0.71 | 3.08 | 2.37 |
| | 32–39 | 3380 | 0.80 | 1.52 | 0.72 |
| | 39–50 | 3735 | 0.82 | 1.68 | 0.86 |
| | 50–57 | 3992 | 0.83 | 2.51 | 1.68 |
| Average | | | 0.81 | 2.00 | 1.19 |
| Group average | | | 0.76 | 1.98 | 1.22 |

H.E., with the shortest period of hemoglobin destruction, 21 days, showed the relatively moderate daily loss of 1.14 mg. of iron. D.A. whose period of hemoglobin breakdown was completed at 29 days, lost 1.56 mg. of iron a day, while D.Sh., whose daily iron loss was the highest of the group, did not attain the minimal blood iron until the fortieth day.

Similarly, no consistent relationship was observed between the rate of hemoglobin destruction and the iron loss. The daily iron loss of D.Sh. was twice as great as that of R.P. yet the rate of decrease of hemoglobin was slower in D.Sh. than in R.P. Again R.P. showed a decreased loss of iron from the body after the blood iron reached its minimum, whereas the iron loss of infants D.A., H.E. and R.D. remained high.

One may conclude from the study of these infants, that a daily loss of approximately 1.25 mg. of iron from the body is to be expected in artificially-fed infants up to 1½ to 2 months of age. Stearns and Stinger ('37), in this laboratory, found that infants over 2 months of age who were given cow's milk feedings, were approximately in iron equilibrium. An artificially-fed baby then during the first 2 months of life may be expected to lose daily approximately 1.25 mg. more of iron than he ingests but to approach equilibrium soon after that period. The total iron lost to the body would thus be from 50 to 75 mg.

The quantities of iron lost daily through the feces by this group of infants are greater than the amounts lost by the breast-fed infants studied by others. The greatest daily loss reported for an infant given human milk was 1.28 mg. (Langstein and Edelstein, '13), a value practically identical with the average daily loss of the babies of this study fed cow's milk. In six of the twenty-six periods studied here, the daily loss exceeded 2 mg. It thus appears that very young infants given cow's milk feedings do not conserve as much of the iron freed by hemoglobin breakdown as do infants fed human milk.

It is obvious that the amount of iron made available for storage by the breakdown of excess hemoglobin will depend upon several related factors, viz., the quantity of blood at

birth and its percentage of hemoglobin, the length of the period of hemoglobin destruction and the level to which the hemoglobin falls, and finally upon the ability of the infant to conserve the iron as it is freed. An infant whose hemoglobin is high at birth and whose period of iron loss is relatively short may be able to store from 250 to 300 mg. of iron from the excess hemoglobin. On the other hand, a premature infant, whose blood volume is small, if the loss of iron continues high for any considerable period, may conserve less than 50 mg. In general, it is probable that the average full-term infant will store from two to three times as much iron from the excess hemoglobin of blood as the maximum amount available in the liver at birth. No infant reported has been able to conserve all of the iron from the excess hemoglobin. If the average iron loss of the infant given cow's milk feedings is from 50 to 75 mg. during the first 2 months of life, it seems that some additional source of iron in the diet is desirable at an early age.

The amount of loss is perhaps more important than the amount stored. The theoretical iron content of the baby of 6 months is approximately equal to its iron content at birth.

## SUMMARY

Alterations of cell volume, erythrocytes, hemoglobin and blood iron of infants under 2 months of age were studied in relation to the excretion of iron during this period.

Blood iron reached its minimal value between 4 and 6 weeks of age. The iron excretion of each infant studied during the period of decreasing blood iron was always greater than the intake. This loss of iron from the body continued for some time after the minimal blood iron was reached. The average daily loss was 1.25 mg., the estimated total loss for the period, from 50 to 75 mg. The one prematurely born infant studied showed as great a loss of iron from the body as did the full-term infants, who presumably had a much larger quantity of excess hemoglobin.

It is concluded that a dietary source of iron is desirable well before 6 months of age.

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# A COMPARATIVE STUDY OF THE RESPIRATORY QUOTIENT FOLLOWING THE INGESTION OF GLUCOSE AND OF FRUCTOSE AS AFFECTED BY THE LACTIC ACID AND CARBON DIOXIDE CHANGES IN THE BLOOD

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TWO FIGURES

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While studies on the respiratory exchange have established with a fair degree of certainty the time, extent and duration of the rise of the respiratory quotient following the ingestion of glucose and of fructose, the significance of the quotient has been called into question by observations on lactic acid and carbon dioxide changes in the blood induced by the sugars. Katayama ('26) observed an increase in blood lactic acid after the ingestion of 1.75 gm. of glucose per kilogram body weight. Similar observations were made by Folch and Formiguera ('34) following the administration of 50 gm. of glucose dissolved in 250 cc. of water. Their data on normal subjects, however, showed an increase of only 0.4 mg., 2.5 mg. and 3.4 mg. %, ½ hour, 1 hour and 2 hours, respectively, after the ingestion of the sugar. Mendel, Engel and Goldscheider ('25), on the other hand, found that the blood lactic acid remained constant after giving by mouth 50 or 70 gm. of glucose; similarly, Oppenheimer ('28) using Mendel and Goldscheider's method for lactic acid analysis, found no increase in the lactic acid of the blood of normal subjects or of patients with diseases of the liver after administration of 50 gm. glucose or of fructose

by mouth. Campbell and Maltby ('28) noted that the ingestion of 100 gm. of fructose was followed by an increase in the lactic acid with a concomitant decrease in the carbon dioxide capacity of the blood, whereas the ingestion of the same amount of glucose had no effect. The oral administration of 50 gm. of glucose to a dog (Taistra, '21) had no effect on the carbon dioxide combining power of the blood. No change in the lactic acid of the blood could be found by Lanyi (cited by Carpenter and Lee, '33) after the administration of glucose to dogs or to man, while the ingestion of fructose was followed by an increase in blood lactic acid, lasting 3 hours. Rose, Giragossintz and Kirstein ('30) injected 25 gm. of glucose or fructose in 125 cc. of water into the small intestine of a dog under amytal anesthesia. The lactic acid in the portal vein rose between 50 and 100% above the resting values in all fructose experiments but showed little or no change with the injection of glucose. The continuous intravenous injection of glucose and of fructose into dogs was found by Wierzuchowski and Laniewski ('31) to lead to an increase in blood lactic acid which was much greater with fructose than with glucose. A rise in the blood lactic acid of rabbits was observed by Koike ('34) after intraperitoneal injections of glucose or fructose.

These experiments would suggest that the respiratory quotient following the ingestion of glucose and of fructose may be affected by the displacement of carbon dioxide from blood bicarbonate as a result of the increase in blood lactic acid. Because of the contradictory observations on lactic acid changes in the blood induced by these sugars, Carpenter and Lee ('33) made simultaneous observations on the respiratory quotient and the carbon dioxide percentage of the alveolar air following the ingestion of glucose and of fructose, to determine whether or not there is a loss of non-metabolic carbon dioxide from the blood. They observed that with a trained subject there was no change in the alveolar carbon dioxide accompanying the rise in the respiratory quotient after the ingestion of either of the two sugars. On the premise,

frequently suggested in the literature, that the constancy of the alveolar air is an indication of true metabolic respiratory quotients, they concluded that no organic acid was formed and that the rise in the respiratory quotient must consequently be regarded as the result of the metabolism of the sugar. As it is questionable whether the constancy of the alveolar carbon dioxide can be regarded as an index of true metabolic quotients, it was deemed advisable to reinvestigate the problem by the more direct method of determining the lactic acid and carbon dioxide content of the blood, and to correlate these findings with the respiratory quotient.

## METHOD

The gaseous exchange was determined by the open circuit method of Carpenter and Fox ('31) with some modifications.[1] The principle of the apparatus used in this method as stated by Carpenter and Fox

is that of the open circuit type in which the expired and inspired air are separated by valves; the expired air passes into a spirometer, from which it is continuously removed by a blower, then passes by a sampling device, and is finally measured by a meter.

The apparatus admits of the measurement of expired air over an extended period, which may be divided into shorter periods of any practical duration. It is adapted to the study of the respiratory exchange both during rest and muscular work, and has a distinct advantage in that the movements of the spirometer bell, which are used to record the character of the breathing on a kymograph paper, take place without the necessity of an increased force of expiration on the part of the subject. The technic of manipulation is easily acquired.

A diagram of the system is presented in figure 1. In our apparatus, provision has been made for the attachment of two sampling bags instead of one, as in the set-up described by Carpenter and Fox. With this arrangement it is possible to

[1] We wish to acknowledge our indebtedness to Doctor Carpenter for his kindly advice at the beginning of the work concerning the construction and use of his respiration apparatus.

cut off one of the sampling bags and to connect the other simultaneously with the main stream of gas flowing through the system.

The original plan was to use basket-ball bladders for collecting the gas samples as suggested by Carpenter and Fox. With this size bag it is necessary to diminish the rate of flow so as to prevent undue distension of the bag and a consequent back pressure which would interfere with aliquot sampling. For this purpose a metal disc[2] with a small opening (G in fig. 1), as recommended by Simonson ('29), was inserted between the main stream of expired air and the sampling bag.

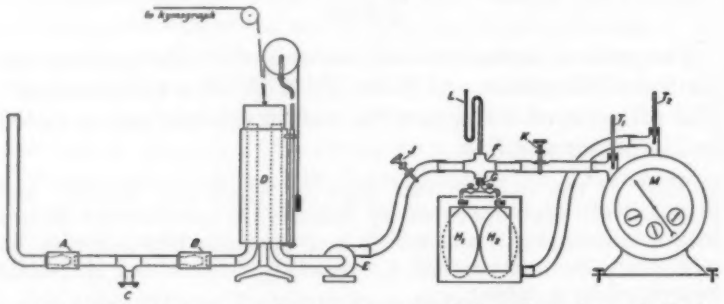

Fig. 1 Schema of the respiration apparatus (after Carpenter and Fox). A, inspiratory valve; B, expiratory valve; C, mouthpiece; D, spirometer; E, rotary blower; F, cross through which expired air passes to the manometer L and into the union G which holds a perforated disc; $H_1$ and $H_2$, sampling bags; M, meter; $T_1$ and $T_2$, thermometers; J and K, screw clamps.

Pressure in the main stream was adjusted by two screw clamps (J and K) as recommended by Carpenter and Fox, thereby regulating the amount of gas collected in the sampling bag.

In checking the apparatus it was found that the pencentage composition of a gaseous mixture is altered when it passes through an aperture less than 1 mm. in diameter. This source of error was discovered by the following procedure: A Tissot spirometer was filled with a mixture of carbon dioxide and oxygen and its exact composition determined by analysis.

[2] Discs with various sized apertures ranging from 0.1 to 0.7 mm. in diameter can be obtained from the Askania-Werke A. G. of Berlin, through their American representative, The American Askania Corporation, Houston, Texas.

The spirometer was then connected to the respiration apparatus and its contents drawn through the system by the blower E (fig. 1). Analysis of the gas mixture that came out of the meter showed that its composition had not been altered in passing through the system; yet the oxygen percentage of the mixture collected in the sampling bag was increased approximately 0.13% and the carbon dioxide percentage decreased approximately 0.20% in passing through an aperture 0.4 mm. in diameter.

The different percentage composition of the gas mixture in the main stream and in the sampling bag after passing through a small aperture, may perhaps be accounted for by the different rates of effusion of oxygen and carbon dioxide. That the different gases have a different rate of effusion is shown by Graham's law which states that, when two different gases are forced through a small aperture, the times $t_1$ and $t_2$ taken for each gas to pass through under a given pressure difference are inversely proportional to the velocities of the gas molecules, according to the formula,

$$\frac{t_1}{t_2} = \frac{u_1}{u_2} = \sqrt{\frac{M_1}{M_2}}$$

where $u_1$ and $u_2$ stand for the average velocities, and $M_1$ and $M_2$, the molecular weights of the two gases.

It is important to note that with alcohol checks it is possible to obtain from gas analyses respiratory quotients close to the theoretical and yet have an appreciable error in the calculated total oxygen consumption and carbon dioxide evolution. In our early work on the method in which we were using a disc with two pin holes which we had made ourselves, alcohol checks yielded respiratory quotients of 0.656, 0.672 and 0.669 which were regarded as fairly satisfactory; the error in the volume of oxygen consumed and carbon dioxide produced, calculated from our analyses of the gas sample was, however, beyond the limit of experimental error. Further experimentation showed that there was an increase of 0.17% in the oxygen and a decrease of 0.16% in the carbon dioxide of the gaseous mixture after it had passed into the sampling bag through the small apertures. If this error had occurred

in the first experiment of table 1 in which are given the results of alcohol checks obtained with a 1.04 mm. aperture, it would have produced only a small change in the respiratory quotient, but a large difference in the oxygen consumption and carbon dioxide production. In this experiment the calculated oxygen consumption was 4.942 liters and carbon dioxide evolution 3.248 liters, giving a respiratory quotient of 0.657. The percentage oxygen and carbon dioxide in the sampling bag (not given in the table) were, respectively, 16.550 and 3.132. If we had had the error referred to above, the oxygen percentage would have been 16.720 and the carbon dioxide percentage 2.972. The customary calculations would then have yielded an oxygen consumption and carbon dioxide production, respectively, of 4.760 and 3.081 liters or a respiratory

TABLE 1

*Alcohol checks with respiration apparatus, using large sampling bags and a disc with an aperture 1.04 mm. in diameter*

| TIME | $C_2H_5OH$ BURNED | AIR PASSED THROUGH SYSTEM | CHANGES IN COMPOSITION OF AIR | | FOUND | | EXPECTED | | ERROR | | R.Q. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $CO_2$ increase | $O_2$ decrease | Total $CO_2$ produced | Total $O_2$ consumed | Total $CO_2$ from combustion of $C_2H_5OH$ | Total $O_2$ consumed in combustion of $C_2H_5OH$ | Total $CO_2$ | Total $O_2$ | |
| *min.* | *gm.* | *liters* | % | % | *liters* | *liters* | *liters* | *liters* | % | % | |
| 15 | 3.371 | 104.385 | 3.112 | 4.734 | 3.248 | 4.942 | 3.280 | 4.920 | —0.97 | +0.45 | 0.657 |
| 15 | 3.306 | 87.281 | 3.646 | 5.518 | 3.182 | 4.816 | 3.216 | 4.825 | —1.06 | —0.19 | 0.661 |
| 15 | 3.322 | 89.442 | 3.580 | 5.406 | 3.202 | 4.835 | 3.232 | 4.848 | —0.93 | —0.27 | 0.663 |

quotient of 0.647 as compared with the respiratory quotient of 0.658 that was actually obtained. The error in the total volume of oxygen consumed, however, would have been 3.7% and in the total volume of carbon dioxide evolved, 5.1%. It was therefore necessary for accurate results to use an aperture larger than 1 mm. in diameter. With an aperture of this size a basket-ball bladder would become filled and distended within a few minutes, thereby necessitating collection of gas samples over correspondingly short intervals. In many metabolism experiments it suffices to collect gas samples over intervals of 15 minutes. After considerable experimentation in which various methods were tried for collecting gas samples, we adopted as the most satisfactory procedure the use of rubber bags large enough to contain approximately 10 liters of gas without exerting a back pressure. A bag of this size

is satisfactory for experiments such as we have been conducting in which the maximum ventilation was from 25 to 30 liters per minute. Using this size bag and a disc with an aperture 1.04 mm. in diameter, we have found in a series of tests with a minimum flow of gas of 6 liters and a maximum of 28 liters per minute, that the bag fills at a uniform rate throughout a 15-minute period. A uniform rate of filling is necessary to make certain that the gas in the sampling bag is an aliquot sample of the gas mixture passing through the system. The bags we used were 46 cm. wide and 55 cm. long. They are prevented from kinking when suspended in the box by two paper clips attached by a wire from the under surface of the top of the box. This arrangement also serves as a safeguard against undue traction by the sampling bag on the rubber tube to which it is cemented.

The rubber sampling bags which we are now using were constructed according to our specifications by a local rubber company.[3] They can, however, be made by any laboratory worker. Two sheets of ordinary cold patching are held together and cut to the desired size and shape. Cold patching 0.046 inch (1.18 mm.) in thickness was used in the bags made for us, but we have found that thinner rubber (0.031 inch, or 0.787 mm.) is satisfactory. When the cold patching has been cut, the partially cured surface of each piece is covered with a thin coat of talc, with the exception of an outer rim about ¾ inch wide. The outer rim having been cleaned with benzol, is covered with a thin layer of rubber cement. A short piece of gum tubing is placed in the proper position to serve as an inlet for the gas and the two sheets of rubber glued together. A small collar is made from the cold patching and a hole cut in it, the exact size of the rubber tubing. The collar is slipped over the tube and fastened by cement to the rim of the bag. A copper tube of 5 mm. internal diameter is fastened into the rubber tubing. A 'gasoline line' nipple and coupling at the free end of the copper tubing serve to make an air-tight connection between the sampling bag and respiration apparatus.

The results obtained with alcohol checks using a disc with a 1.04 mm. aperture and large rubber bags for collecting the samples are given in table 1. The accuracy of the method has been further tested by simulating experimental conditions in which alterations occur in the oxygen and carbon dioxide

[3] The Holfast Rubber Company, Atlanta, Ga.

percentage of the expired air passing through the system. A Tissot spirometer was connected by a T-tube with the respiration apparatus. A rubber tube led off from the arm of the T-tube to a calibrated 8 liter aspirator bottle. The gas in this bottle could be displaced and forced into the T-tube by introducing acidulated water from another aspirator bottle, the flow of water being controlled by a stopcock interposed in the tubing connecting the two bottles. The Tissot spirometer and aspirator bottle were filled with different gas mixtures, the exact composition of which was determined by analysis. The flow of gas from either of the containers could be interrupted at will by clamping the rubber tubing near the spirometer or aspirator bottle. A flutter valve placed in the path of flow between the Tissot spirometer and T-tube prevented the gas from the aspirator bottle flowing backward into the spirometer. Gas was first delivered from the Tissot spirometer, then from the aspirator bottle and again from the spirometer, thereby altering in the course of the experiment the percentage composition of the gas flowing through the system. During the first and last few minutes of the experiment, gas was delivered from the spirometer so that the percentage composition of the gas in the system was the same at the end as at the beginning of the experiment. As the total volume and percentage composition of the gas mixture were known, it was an easy matter to determine the exact amount of oxygen and carbon dioxide that had passed through the system. The total oxygen and carbon dioxide were then calculated from the oxygen and carbon dioxide percentage of the gas mixture in the sampling bag in the same manner as in a metabolism experiment. The volume of the two gases thus calculated was almost identical with the known volume that had passed through the system. In most instances the difference was less than $\frac{1}{2}\%$. The results of this experiment are given in table 2.

The sample of gas for analysis is drawn from the large rubber bag into a well oiled 50 cc. Vim syringe. Connection between the sampling bag and syringe is made by slipping a

TABLE 2

*Checks on respiration apparatus using large sampling bag and disc with 1.04 mm. aperture. Gas mixtures of different percentage composition were delivered alternately from two containers*

| No. | KNOWN GAS MIXTURES PASSED THROUGH THE APPARATUS | | | | | | | | | | FOUND | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 103.55 liters delivered from large spirometer | | | | 7.00 liters delivered from small container | | | | Total volume delivered from the two containers | | Sample in bag | | Total volume of gas as calculated from sample | | Error in total volume | |
| | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ |
| | % | % | *liters* | *liters* | % | % | *liters* | *liters* | *liters* | *liters* | % | % | *liters* | *liters* | % | % |
| 1 | 19.364 | 2.405 | 20.051 | 2.490 | 17.158 | 5.393 | 1.201 | 0.378 | 21.253 | 2.867 | 19.169 | 2.585 | 21.191 | 2.858 | —0.288 | —0.356 |
| 2 | 19.405 | 2.435 | 20.094 | 2.521 | 15.666 | 10.093 | 1.097 | 0.707 | 21.191 | 3.228 | 19.150 | 2.903 | 21.170 | 3.209 | —0.100 | —0.580 |
| 3 | 19.007 | 2.972 | 19.682 | 3.078 | 16.229 | 8.307 | 1.136 | 0.582 | 20.818 | 3.659 | 18.856 | 3.260 | 20.845 | 3.604 | +0.130 | —1.500 |

rubber tube fastened to the syringe, over the copper tube leading into the bag. The gas is 'pumped' back and forth into the bag several times so as to mix thoroughly the small amount of air in the dead space of the rubber tube with the gas in the bag. The syringe is then filled and the rubber tube closed tight by means of a screw clamp. Two or 3 cc. of mercury in the bottom of the syringe serve as an extra precaution against escape of the gas, while the syringes are standing in a rack until the gases are analyzed. The volume of the gas in the bag is measured by displacement of water from an 8 liter calibrated aspirator bottle. This volume is added to the amount of gas that has passed through the meter.

## EXPERIMENTAL PROCEDURE

The subjects of these experiments, three male adults, weighing 80, 70 and 55 kilo., respectively, came to the laboratory in the fasting state, between 7.15 and 7.45 in the morning, the last meal having been taken not later than 7 o'clock the evening before. Upon arriving at the laboratory the subject reclined on a couch for 30 minutes to recover from the effects of previous exertion, which he had endeavored to keep at a minimum. After this preliminary rest period, the mouth-piece commonly employed in metabolism experiments was inserted, and the subject then breathed into the respiratory apparatus for 45 minutes, during which time the 'post-absorptive' gaseous exchange was determined, gas samples being collected over 15-minute period intervals. At the conclusion of the post-absorptive period a sample of venous blood was drawn from the arm without stasis. A portion of the blood was then delivered from the syringe into chilled test tubes under oil for determinations of its carbon dioxide content. Coagulation was prevented by the addition of 1 mg. potassium oxalate per cubic centimeter of blood. The remainder of the blood was used for lactic acid analysis. Glycolysis was inhibited in this portion of the blood by the addition of 10 mg. of sodium fluoride per cubic centimeter of blood. The samples for carbon dioxide determinations were surrounded by ice, and the analyses

usually completed within 2 to 3 hours after withdrawal of the blood. Preliminary tests had shown that there was no loss of carbon dioxide during this time when the blood was kept under these conditions.

The gas analyses were made by two analysts using the Haldane-Henderson method. Checks within 0.02% were required. The lactic acid of the blood was determined by the Friedemann, Cotonio and Shaffer ('27) method, and its carbon dioxide content by the Van Slyke procedure (Peters and Van Slyke, '32).

After the withdrawal of the post-absorptive blood sample, 500 cc. water, or 50 gm. glucose, 50 gm. fructose or 25 gm. each of glucose and fructose in 500 cc. of water at 37°C. were ingested, the experiments with water serving as controls. The subject then resumed the recumbent position and the determination of the gaseous exchange proceeded in the same manner as during the post-absorptive period. Gas samples were collected over 15-minute periods, except in a few experiments to be referred to later, in which the intervals were shorter. Blood samples were drawn at various times. An attempt was made to obtain the sample at the end of that 15-minute period which we had found by previous experience usually showed the highest respiratory quotient. In this we were only partially successful as was to be expected, since the highest respiratory quotient was not always obtained in the same 15-minute period; in the glucose experiments, the maximum quotient was observed sometimes in the third, sometimes in the fourth 15-minute period, whereas in the fructose experiments the highest quotient was obtained sometimes in the second and sometimes in the third period.

## RESULTS

As seen in table 3, the control experiments with water carried out in the same way as the sugar experiments, showed no increase in the lactic acid or decrease in the carbon dioxide content of the blood after the ingestion of 500 cc. water. In one experiment in which the lactic acid remained constant,

TABLE 3

*Lactic acid and carbon dioxide content of the blood, before and after ingestion of water (control experiments), glucose and fructose*

| EXPERIMENT NO. | SUBSTANCE INGESTED | NON PROTEIN R.Q.[2] | | BLOOD LACTIC ACID | | $CO_2$ CONTENT OF BLOOD | | 15 MINUTES POST-INGESTION PERIOD[1] IN WHICH SECOND BLOOD SAMPLE WAS DRAWN |
|---|---|---|---|---|---|---|---|---|
| | | Post-absorptive | Period[1] before drawing second blood sample | End of post-absorptive period[1] | Second sample | End of post-absorptive period[1] | Second sample | |
| | | | | *mg. %* | | *volume %* | | |
| 1 | 500 cc. water | 0.83 | 0.82 | .... | .... | 57.6 | 58.0 | End of 3rd |
| 2 | 500 cc. water | 0.85 | 0.83 | 11.2 | 10.4 | 60.0 | 62.0 | End of 3rd |
| 3 | 500 cc. water | 0.84 | 0.84 | 10.8 | 10.7 | 61.6 | 61.0 | End of 3rd |
| 4 | 500 cc. water | 0.81 | 0.79 | 8.7 | 7.9 | 57.8 | 57.5 | End of 3rd |
| 5 | 50 gm. glucose | 0.78 | 0.78 | 10.3 | 7.4 | 54.6 | 54.1 | End of 3rd |
| 6 | 50 gm. glucose | 0.78 | 0.83 | 14.6 | 15.3 | 60.2 | 56.7 | End of 3rd |
| 7 | 50 gm. glucose | 0.76 | 0.85 | 9.4 | 9.4 | .... | .... | End of 3rd |
| 8 | 50 gm. glucose | 0.75 | 0.85 | 14.6 | 14.1 | 59.8 | 59.8 | End of 3rd |
| 9 | 50 gm. glucose | 0.78 | 0.86 | 8.9 | 9.8 | 54.9 | 55.3 | End of 3rd |
| 10 | 50 gm. glucose | 0.80 | 0.90 | 6.3 | 11.0 | 58.9 | 54.9 | End of 4th |
| 11 | 50 gm. fructose | 0.80 | 1.05 | 9.3 | 14.5 | 60.1 | 54.1 | End of 2nd |
| 12 | 50 gm. fructose | 0.78 | 0.96 | 13.8 | 25.8 | 60.6 | 53.6 | End of 2nd |
| 13 | 50 gm. fructose | 0.79 | 0.95 | 7.3 | 16.5 | 56.1 | 52.0 | End of 3rd |
| 14 | 50 gm. fructose | 0.78 | 0.95 | 13.8 | 21.2 | 56.0 | 54.0 | End of 3rd |
| 15 | 50 gm. fructose | 0.82 | 0.98 | 9.3 | 14.7 | .... | .... | End of 3rd |
| 16[3] | 50 gm. fructose | 0.77 | 0.86 | 9.1 | 16.2 | 58.6 | 54.5 | End of 1st |
| 17[3] | 50 gm. fructose | 0.78 | 0.96 | 10.8 | 15.2 | 61.9 | 58.6 | End of 1st |
| 18 | 25 gm. glucose 25 gm. fructose | 0.78 | 0.88 | .... | .... | 60.1 | 58.3 | End of 4th |
| 19 | 25 gm. glucose 25 gm. fructose | 0.77 | 0.96 | 8.6 | 15.5 | 56.7 | 56.4 | End of 2nd |
| 20 | 25 gm. glucose 25 gm. fructose | 0.76 | 1.03 | 20.8 | 33.0 | 59.2 | 54.4 | End of 2nd |
| 21 | 25 gm. glucose 25 gm. fructose | 0.76 | 0.93 | 7.5 | 15.9 | 56.5 | 55.9 | End of 3rd |
| 22 | 25 gm. glucose 25 gm. fructose | 0.75 | 0.87 | 17.8 | 26.9 | 59.6 | 56.1 | End of 3rd |
| 23 | 25 gm. glucose 25 gm. fructose | 0.78 | 1.02[4] | 9.1 | 19.9 | 59.8 | 55.1 | Middle of 3rd |

[1] Periods were of 15 minutes duration.

[3] A third blood sample drawn at the middle of the fourth 15-minute period contained 24.4 mg. % lactic acid and 52.0 volumes % carbon dioxide. The non-protein respiration quotient for the 8-minute period immediately before the withdrawal of the blood was 1.04.

[3] A third blood sample, drawn at the middle of the third 15-minute period, contained 20.6 mg. % lactic acid and 54.0 volumes % carbon dioxide. The non-protein respiratory quotient for the 7-minute period, immediately before the withdrawal of the blood, was 1.08.

[4] Ten-minute period.

[2] The urine was analyzed for nitrogen in each experiment for the calculation of the non-protein respiratory quotient.

there was an increase in the carbon dioxide content of the second blood sample. We are at a loss to account for this irregularity as perfect checks were obtained on the carbon dioxide analyses. It is possible that in spite of the precautions observed there was a slight stasis during the withdrawal of the second sample.

In the glucose experiments, the blood lactic acid remained constant in five out of six experiments; one showed an increase of 4.7 mg. % with a concomitant decrease of 5 volumes % in the carbon dioxide content. The blood sample in this experiment was drawn 15 minutes later than in the others. In the remainder of the experiments, the carbon dioxide content of the blood remained constant except in one instance (experiment no. 6) when it fell 3.5 volumes % although there was no change in the blood lactic acid. Irregularities of this nature are found also in the results reported by Campbell and Maltby ('28).

In the fructose experiments, there was in each instance an increase in the lactic acid of the blood drawn 30 to 45 minutes after the ingestion of the sugar, and a uniform decrease in the carbon dioxide content of the blood. The decrease in the carbon dioxide content was greater than one might expect from a complete reaction of lactic acid with bicarbonate since 2.5 cc. of carbon dioxide would have been displaced by each 10 mg. of lactic acid entering into the reaction. This suggests that there may have been some other fixed acid or acids formed as intermediate products in the metabolism of fructose.

After the ingestion of the mixture of the sugars there was an increase in the blood lactic acid in all the experiments and a decrease in the carbon dioxide content of the blood in all but two experiments. Since there was no increase in the lactic acid in the glucose experiments, the increase induced by the mixture of the sugars must be attributed to the action of fructose. It is interesting to note that the average lactic acid and carbon dioxide changes of the blood were approximately the same after the ingestion of 50 and 25 gm. of fructose.

It should be noted that the lactic acid increase occurred within a short time after the ingestion of fructose. In experiments 16 and 17 the lactic acid had risen 7.1 and 4.4 mg., respectively, 15 minutes after ingestion of the sugar while the carbon dioxide had fallen 4.1 and 3.3 volumes % (fig. 2). During this time, the respiratory quotient had risen to 0.85 and

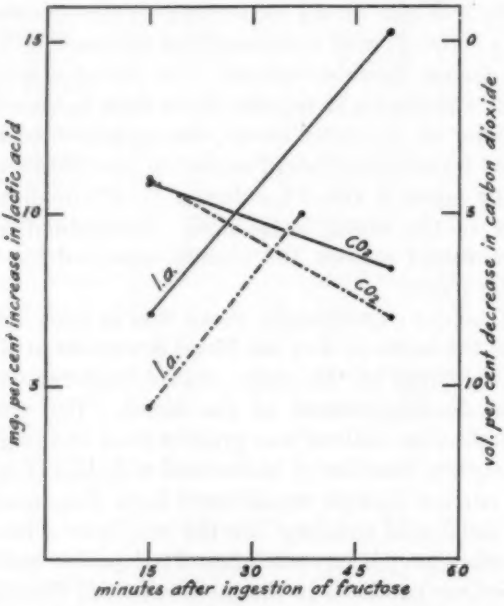


Fig. 2 Changes in the lactic acid and carbon dioxide of the blood in two subjects after the ingestion of fructose.

0.92, respectively, which was an increase of 0.11 and 0.13 over the post-absorptive level. In the same period after the ingestion of glucose the average rise of the respiratory quotient was only 0.01 or 0.02.

From these results, it is obvious that the character of the metabolism of fructose is masked by the blowing off of non-metabolic carbon dioxide. In order then to interpret the

findings of the respiratory exchange correctly and to draw a comparison between the respiratory quotients obtained after the ingestion of glucose and of fructose, it becomes necessary to correct the respiratory quotients of the fructose experiments by making proper allowance for the volume of non-metabolic carbon dioxide blown off.

The correction of the respiratory quotients obtained for the time elapsing between the ingestion of the sugar and the drawing of the blood sample has been made in two ways: 1) By deducting from the total carbon dioxide eliminated the amount that would be liberated by the lactic acid that was formed after the ingestion of fructose, 2) by deducting an amount equivalent to the loss of carbon dioxide from the blood as calculated from the reduction in its carbon dioxide content. The results of these calculations in the form of 'corrected' respiratory quotients are given in table 4. The total volume of blood has been estimated as 7% of the body weight of the subject and 1 mg. of lactic acid taken as the equivalent of 0.25 cc. of carbon dioxide. The calculations were made on the assumption that there was a complete reaction between the lactic acid and bicarbonate of the blood. This perhaps is too generous an allowance, for Mellanby and Thomas ('20–'21) found that the addition of lactic acid to blood in vitro diminishes its carbon dioxide capacity by less than 50% of the quantity demanded by complete reaction with bicarbonate. Similar results were also obtained by Evans ('22). In the experiments of Gesell, Krueger, Gorham and Bernthal ('30) it appears that approximately 40% only of the lactic acid formed during the administration of low oxygen, combined with the bicarbonate of the blood. By making the maximum allowance for displacement of carbon dioxide by the increased lactic acid, the question is therefore put to the most severe test. If our calculations had been made on the assumption that only 40 to 50% of the lactic acid reacted with bicarbonate, there would have resulted a considerably smaller difference between the corrected and actual respiratory quotients.

The respiratory quotients in the last column of table 4 represent the average quotients obtained from a number of experiments on the different subjects after the administration

TABLE 4

*Respiratory quotients after fructose ingestion, corrected for blowing off of non-metabolic carbon dioxide, and compared with respiratory quotients obtained with glucose over corresponding periods of time*

| EXPERIMENT NO. | | TIME BETWEEN INGESTION OF SUGAR AND DRAWING OF BLOOD SAMPLE | INCREASE IN BLOOD LACTIC ACID | DECREASE IN $CO_2$ CONTENT OF THE BLOOD | NON-PROTEIN RESPIRATORY QUOTIENT | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Obtained for time between ingestion of sugar and drawing of blood sample | Corrected for $CO_2$ equivalent of lactic acid increase | Corrected for $CO_2$ equivalent of decrease in blood $CO_2$ | Of corresponding period in glucose experiments |
| | | *minutes* | *mg. %* | *vol. %* | | | | |
| 16 | (Increase over basal) | 15 | 5.0 | 4.2 | 0.864 | 0.851 (0.086)[1] | 0.826 (0.061) | 0.777 (0.011) |
| 11 | (Increase over basal) | 30 | 5.2 | 6.0 | 0.932 | 0.920 (0.122) | 0.875 (0.077) | 0.822 (0.033) |
| 15 | (Increase over basal) | 30 | 12.0 | 7.0 | 0.948 | 0.938 (0.166) | 0.914 (0.142) | 0.760 (0.000) |
| 17 | (Increase over basal) | 30 | 4.5 | 3.3 | 0.931 | 0.908 (0.128) | 0.880 (0.100) | 0.760 (0.000) |
| 12 | (Increase over basal) | 45 | 9.2 | 4.1 | 0.853 | 0.843 (0.114) | 0.836 (0.107) | 0.808 (0.052) |
| 13 | (Increase over basal) | 45 | 7.4 | 2.0 | 0.907 | 0.899 (0.121) | 0.898 (0.120) | 0.808 (0.052) |

[1] The post-absorptive quotients (not included in the table) can be obtained by subtracting the increase over the basal enclosed in parentheses from the figure immediately above.

of glucose, except that of experiment 17 which is the respiratory quotient of only one experiment on this subject with glucose. The respiratory quotients of the glucose and those of the fructose experiments with which they are compared, were obtained from the respiratory exchange of the same

subject, over the same length of time and under similar experimental conditions.

It will be observed in the table that after the maximum allowance has been made for the blowing off of non-metabolic carbon dioxide in the fructose experiments, the respiratory quotients for 15-, 30- and 45-minute periods after ingestion of the sugar, are still higher than those obtained in the glucose experiments over the same length of time. The increase in the respiratory quotient over that of the post-absorptive periods is given in the table in parentheses. The post-absorptive quotients, which are not included in the tables, can be obtained by subtracting the increase enclosed in parentheses from the figure immediately above. From these observations we may conclude that the difference between the respiratory quotients observed after the ingestion of glucose and of fructose cannot be entirely accounted for by the formation of acids and elimination of the non-metabolic carbon dioxide that follow the ingestion of fructose. The difference must lie either in the combustion of relatively more carbohydrate during the first 45 minutes after the ingestion of fructose or in the transformation of this sugar into fat.

Respiratory quotients above unity which have been observed by a number of workers after the ingestion of fructose, have been offered as evidence of the conversion of this sugar into fat. Conceivably, these quotients might be reduced to unity or below when a correction is made for the blowing off of non-metabolic carbon dioxide. The ideal procedure would be to draw the blood sample at the time when the respiratory quotient is above unity. Such was our original plan at the outset of these experiments, as it was believed that the high respiratory quotients we had observed in earlier experiments could be easily duplicated. Unfortunately, these high quotients did not recur except in a few experiments (nos. 11, 16, 17, 20 and 23 of table 1). In two of these experiments (16 and 17), the high quotients were obtained by collecting the gas sample over 5- and 7-minute periods, respectively, instead of the usual 15-minute period.

The experiments with respiratory quotients above unity are given in table 5. This table gives also the quotients derived by correcting the original quotients for the carbon dioxide equivalent of blood lactic acid, and for the equivalent of the decrease in carbon dioxide content of the blood. In experiments 16 and 17 three blood samples were taken. In both experiments, the first sample was drawn at the end of the

TABLE 5

*Respiratory quotients above unity corrected for non-metabolic carbon dioxide in the expired air*

| EXPERIMENT NO. | SUGAR INGESTED | TIME (AFTER INGESTION) OVER WHICH R.Q. WAS DETERMINED | NON-PROTEIN RESPIRATORY QUOTIENT | | |
|---|---|---|---|---|---|
| | | | Obtained from gaseous exchange | Corrected for $CO_2$ equivalent of increase in blood lactic acid | Corrected for $CO_2$ equivalent of decrease in blood $CO_2$ |
| | | *minutes* | | | |
| 16 | 50 gm. fructose | 37 to 45 | 1.040 | 1.031 | 1.023 |
| 17 | 50 gm. fructose | 30 to 37 | 1.075 | 1.059 | 1.020 |
| 11 | 50 gm. fructose | 30 to 45 | 1.050 | 1.011 | 0.997 |
| 20 | 25 gm. glucose<br>25 gm. fructose | 25 to 30 | 1.094 | 1.070 | 1.054 |
| 23 | 25 gm. glucose<br>25 gm. fructose | 25 to 35 | 1.019 | 1.005 | 0.986 |
| 61 | 50 gm. fructose | 15 to 30 | 1.052 | 1.042 | 1.005 |
| 61 | 50 gm. fructose | 30 to 45 | 1.088 | 1.073 | 1.019 |
| 62 | 50 gm. fructose | 30 to 45 | 1.067 | 1.055 | 1.010 |
| 63 | 25 gm. glucose<br>25 gm. fructose | 15 to 30 | 1.073 | 1.062 | 1.021 |
| 64 | 25 gm. glucose<br>25 gm. fructose | 45 to 60 | 1.068 | 1.054 | 1.011 |
| 65 | 25 gm. glucose<br>25 gm. fructose | 45 to 60 | 1.056 | 1.045 | 1.005 |

post-absorptive periods and the second sample 15 minutes after the ingestion of the fructose. In experiment 16 the third sample was taken 30 minutes, and in experiment 17, 22 minutes after the first sample. As seen in figure 2, these experiments indicate that the formation of lactic acid and the decrease in the carbon dioxide of the blood began shortly after the ingestion of the sugar and continued at a fairly uniform rate.

In experiments 11, 20 and 23, only two blood samples were taken, one at the close of the post-ingestion periods and the other 30 to 45 minutes after ingestion of the sugar. On the basis of the observations in experiments 16 and 17, all calculations of the increase in lactic acid, or decrease in carbon dioxide content of the blood for the time over which the respiratory quotient was determined were made on the assumption of a uniform rate of change.

It will be observed that in every instance the corrected respiratory quotients still remain above unity. The correction for lactic acid formation lowers the quotient to a slighter extent than that for the carbon dioxide decrease of the blood. This is in accordance with the observation mentioned previously that the decrease in the carbon dioxide of the blood was greater than could be accounted for by the rise in lactic acid.

In experiments carried out before the present investigation was undertaken, respiratory quotients above unity had been obtained several times after the ingestion of fructose and a mixture of glucose and fructose. Since, however, the same subject served in the present series, the early experiments (nos. 61 to 65) showing respiratory quotients above unity, have been incorporated in table 5. The correction of the quotients was made on the assumption that the quantitative changes in the lactic acid and carbon dioxide content of the blood were approximately the same, and occurred at the same rate as subsequently observed in other experiments on this subject after the ingestion of fructose. The quotients thus corrected as in the case of the experiments of the present series, are still above unity.

## CONCLUSIONS

The respiratory quotients obtained within 45 minutes after the ingestion of 50 gm. fructose are not true metabolic quotients, for, as shown in these experiments there is a gradual rise in blood lactic acid and a decrease in the carbon dioxide content of the blood, beginning within a short time after the ingestion of the sugar. When, however, correction was made

for the non-metabolic carbon dioxide in the expired air, the respiratory quotients obtained after the ingestion of fructose in a number of experiments were still above unity. It must therefore be concluded that a portion of the ingested fructose was converted into fat.

As regards glucose, it would appear from our experiments that the respiratory quotients obtained over at least the first 30 minutes after the ingestion of the sugar, are true metabolic quotients.

## SUMMARY

A study has been made of the respiratory quotient correlated with blood lactic acid and the carbon dioxide content of the blood, following the ingestion of 50 gm. glucose, 50 gm. fructose and a mixture of 25 gm. glucose and 25 gm. fructose.

The respiratory exchange was determined by the open circuit method of Carpenter and Fox. It was found that an error was introduced by using discs with a small aperture (less than 1 mm. in diameter), as recommended by Simonson, for collecting aliquot samples of the expired air. The percentage composition of the gas was altered in passing through the small aperture; the gaseous mixture in the sampling bag had a higher oxygen and lower carbon dioxide percentage than that of the main gaseous stream passing through the system. When the gases passed through an aperture 1.04 mm. in diameter, the percentage composition of the mixture in the sampling bag was identical with that of the main stream.

Large rubber bags, that contain approximately 10 liters of gas without exerting a back pressure, proved satisfactory for collecting gas samples through an aperture 1.04 mm. in diameter. A detailed account is given of the construction of the rubber bags used in these experiments.

In five experiments in which 50 gm. glucose was ingested there was generally no increase in the lactic acid nor decrease in the carbon dioxide content of the blood. It was concluded that the respiratory quotients obtained within 30 minutes after the ingestion of glucose were true metabolic quotients.

The ingestion of the same quantity of fructose led in each instance, within 15 minutes and later, to an increase in blood lactic acid and an attendant decrease in the carbon dioxide content of the blood. The same events followed the ingestion of a mixture of 25 gm. glucose and 25 gm. fructose. The average lactic acid and carbon dioxide changes observed with this mixture, were approximately of the same magnitude as those observed with 50 gm. fructose. From these observations, it is evident that the respiratory quotient obtained after the ingestion of fructose is not a true metabolic quotient.

When allowance was made for the blowing off of non-metabolic carbon dioxide, the respiratory quotients obtained over a period of 30 to 45 minutes after the ingestion of fructose, were considerably higher than the quotients obtained over the corresponding time after the ingestion of glucose. The difference indicates either a combustion of relatively more carbohydrate after the ingestion of fructose, or a transformation of this sugar into fat.

When the respiratory quotients obtained with fructose were above unity and these high quotients were corrected for the non-metabolic carbon dioxide, as calculated from the increase in the lactic acid and from the decrease in the carbon dioxide content of the blood, they still remained above unity. It is therefore concluded that a portion of the ingested fructose was converted into fat.

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# EFFECT OF DIET ON THE CONSTANCY OF THE URINARY NITROGENOUS CONSTITUENTS EXCRETED DAILY BY PRE-SCHOOL CHILDREN [1]

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ONE FIGURE

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Although investigators have determined the distribution of nitrogen in the urine, few studies have been made on normal pre-school children who have received constant diets for long periods of time. The two metabolic studies conducted in this laboratory on six normal pre-school children (Hawks, Bray and Dye, '37) included daily urinary analysis for total nitrogen, urea, uric acid, ammonia, creatine, creatinine and amino acid nitrogen. There were two children in the first study and four in the second. This paper presents the data obtained, following a 10- or 12-day preliminary period, during 21 consecutive days when the children received diets containing 3 gm. of protein per kilogram of body weight, and also the data obtained during the following 15 or 24 days when they received diets containing 4 gm. of protein per kilogram. Thus, the data show, first, the daily variations in the urinary nitrogenous substances when the children received a constant diet; second, the variations which occurred when the protein content of the diet was increased; third, the length of time necessary for the children to adjust to the change in diet; and fourth, the individual differences among the children.

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The complete experimental procedure was described in detail in an earlier communication (Hawks, Bray and Dye, '37). In order to prevent changes in the acidity and in the distribution of the urinary nitrogenous substances, the urine samples were all kept on ice until the 24-hour sample was complete. They were then taken to the laboratory, adjusted to room temperature, made to a definite volume and analyzed immediately for acidity, urea, ammonia, uric acid and amino acid nitrogen. The other determinations were made as soon as possible.

Total nitrogen was determined by the official Kjeldahl method (Association of Official Agricultural Chemists, '25) and uric acid, amino acid, creatine and creatinine according to the methods described by Folin ('22). In the first study, urea and ammonia were determined by the colorimetric method and, in the second study, by the aeration method. All the data reported are averages of duplicate or triplicate determinations which checked within 2%.

## RESULTS

Table 1 gives a statistical interpretation of the daily variation in the 24-hour urinary excretion for each child when he received a constant medium protein diet. On account of the large volume of data, only the range in values and the mean with its probable error are given, together with the standard deviation and coefficient of variation.

In the second experiment, the lower range in urine volume as well as a reduction in the coefficients of variation from a high value of 11.2 to 8.9 indicates that the volume was more constant than in the first experiment. This is not surprising since the water intake was more carefully controlled during the second experiment. In spite of the changes in volume the specific gravity remained exceedingly constant, the coefficients of variation for five of the six children being 0.1.

Since the composition of identical diets varied somewhat from period to period and even between duplicate diets weighed on the same day (Hawks, Bray and Dye, '37), the

TABLE 1

*Daily variation in 24-hour urinary excretion on a constant 3-gm. protein diet*

| FUNCTION | CHILD | EXPERIMENT | RANGE | MEAN | STANDARD DEVIATION | COEFFICIENT OF VARIATION |
|---|---|---|---|---|---|---|
| | | | | | | % |
| Volume (cc.) | D | I | 820–1225 | 1034±17.0 | 115.6 | 11.2 |
| | B | | 835–1330 | 1070±17.3 | 117.5 | 11.0 |
| | J | II | 585– 800 | 681± 9.6 | 60.4 | 8.9 |
| | C | | 690– 970 | 800±10.3 | 68.6 | 8.6 |
| | V | | 870–1130 | 998± 7.5 | 50.8 | 5.1 |
| Specific gravity | D | I | 1.010–1.015 | 1.0131±0.0002 | 0.0014 | 0.1 |
| | B | | 1.012–1.017 | 1.0140±0.0002 | 0.0015 | 0.1 |
| | J | II | 1.008–1.013 | 1.0106±0.0002 | 0.0014 | 0.1 |
| | C | | 1.008–1.013 | 1.0105±0.0003 | 0.0018 | 0.2 |
| | V | | 1.007–1.012 | 1.0095±0.0002 | 0.0012 | 0.1 |
| Total nitrogen (gm.) | D | I | 7.09–7.80 | 7.46±0.03 | 0.22 | 2.9 |
| | B | | 6.92–8.24 | 7.65±0.05 | 0.33 | 4.3 |
| | J | II | 4.53–5.18 | 4.93±0.03 | 0.17 | 3.4 |
| | C | | 5.40–5.98 | 5.78±0.02 | 0.14 | 2.4 |
| | V | | 6.31–6.96 | 6.71±0.03 | 0.19 | 2.8 |
| Urea nitrogen (gm.) | D | I | 6.30–6.99 | 6.65±0.03 | 0.21 | 3.2 |
| | B | | 5.83–7.40 | 6.70±0.05 | 0.33 | 4.9 |
| | J | II | 3.91–4.53 | 4.31±0.02 | 0.15 | 3.5 |
| | C | | 4.55–5.21 | 4.95±0.02 | 0.16 | 3.2 |
| | V | | 5.40–6.04 | 5.75±0.02 | 0.17 | 2.9 |
| Ammonia nitrogen (mg.) | D | I | 185–279 | 226±3.8 | 25.5 | 11.3 |
| | B | | 255–408 | 323±6.4 | 43.6 | 13.5 |
| | J | II | 92–125 | 110±1.6 | 10.0 | 9.1 |
| | C | | 135–199 | 158±2.4 | 15.8 | 10.0 |
| | V | | 141–194 | 168±2.5 | 16.7 | 10.0 |
| Acidity (cc. 0.1 N acid) | D | I | 170–229 | 199±2.4 | 16.2 | 8.2 |
| | B | | 206–291 | 247±3.3 | 22.4 | 9.1 |
| | J | II | 63–108 | 85±2.3 | 14.6 | 17.2 |
| | C | | 77–116 | 95±1.6 | 10.8 | 11.5 |
| | V | | 83–140 | 112±2.1 | 14.4 | 12.8 |
| Uric acid nitrogen (mg.) | D | I | 75– 98 | 86±1.0 | 6.6 | 7.7 |
| | B | | 94–122 | 110±1.0 | 7.1 | 6.5 |
| | J | II | 69– 77 | 73±0.4 | 2.5 | 3.4 |
| | C | | 87–106 | 95±0.7 | 4.6 | 4.9 |
| | V | | 97–113 | 107±0.6 | 4.3 | 4.1 |
| Amino acid nitrogen (mg.) | D | I | 49– 74 | 63±1.0 | 7.1 | 11.4 |
| | B | | 56– 90 | 69±1.4 | 9.5 | 13.8 |
| | J | II | 54– 87 | 70±1.6 | 10.2 | 14.6 |
| | C | | 68–109 | 87±1.9 | 12.6 | 14.4 |
| | V | | 74–120 | 97±2.0 | 13.4 | 13.8 |
| Creatinine nitrogen (mg.) | D | I | 126–140 | 134±0.5 | 3.5 | 2.6 |
| | B | | 131–148 | 141±0.7 | 4.8 | 3.4 |
| | J | II | 70– 78 | 75±0.4 | 2.3 | 3.1 |
| | C | | 83– 91 | 87±0.4 | 2.6 | 3.0 |
| | V | | 104–114 | 109±0.4 | 2.7 | 2.5 |
| Creatine nitrogen (mg.) | D | I | 90–118 | 108±1.0 | 6.5 | 6.1 |
| | B | | 118–141 | 126±0.8 | 5.7 | 4.6 |
| | J | II | 74– 86 | 82±0.6 | 3.6 | 4.4 |
| | C | | 75–100 | 92±1.1 | 7.0 | 7.6 |
| | V | | 86–110 | 98±1.0 | 7.1 | 7.2 |

nitrogen intake for each child probably varied not only every period, but also on different days during a period. On account of these differences, one would expect the coefficients of variation for the urinary nitrogenous constituents to be at least as great as those found for intake in the two experiments which were 2.7 and 2.3. Table 1 shows that the coefficient of variation for total nitrogen for subject D was practically the same as that for the diet during the first experiment, while the coefficient for B was 4.3. In the second experiment, the values for J were less consistent than the figures for diet but those for the other two children varied to about the same extent. The increase in variability is in reality extremely small when one considers the number of uncontrollable biological errors, such as those connected with the collection of samples, the physical state of the child, exercise, and psychological reactions. Figure 1, which illustrates the daily fluctuations in the diet nitrogen in relation to the total urinary nitrogen excreted by each child, also indicates that the fluctuations for urinary nitrogen seem to be in somewhat the same proportion and not much greater than diet variations. Therefore, the data seem to indicate that the total urinary nitrogen excretion of the children had reached an equilibrium similar to or even equal to that for diet nitrogen.

The coefficients of variation for urea nitrogen, which were between 2.9 and 4.9, were slightly greater than those for total nitrogen. The increase, however, was probably insignificant. On the other hand, the values for ammonia nitrogen showed much greater variations, the coefficients ranging from 9.1 to 13.5. Since the urine specimens were kept on ice at all times and since the determinations were made as soon as the specimens were complete it scarcely seems possible that the increased variation was entirely due to the fluctuations in the decomposition of the urea although this may have been a determining factor. Since the values of ammonia varied to such an extent, it is not surprising that those for total titratable acidity varied in somewhat the same degree. The coefficients were lower in the first experiment, but higher in the second and ranged from 8.2 to 17.2.

DAILY VARIATION IN TOTAL URINARY NITROGEN AS RELATED TO A CONSTANT AND ALSO TO AN INCREASE IN NITROGEN INTAKE

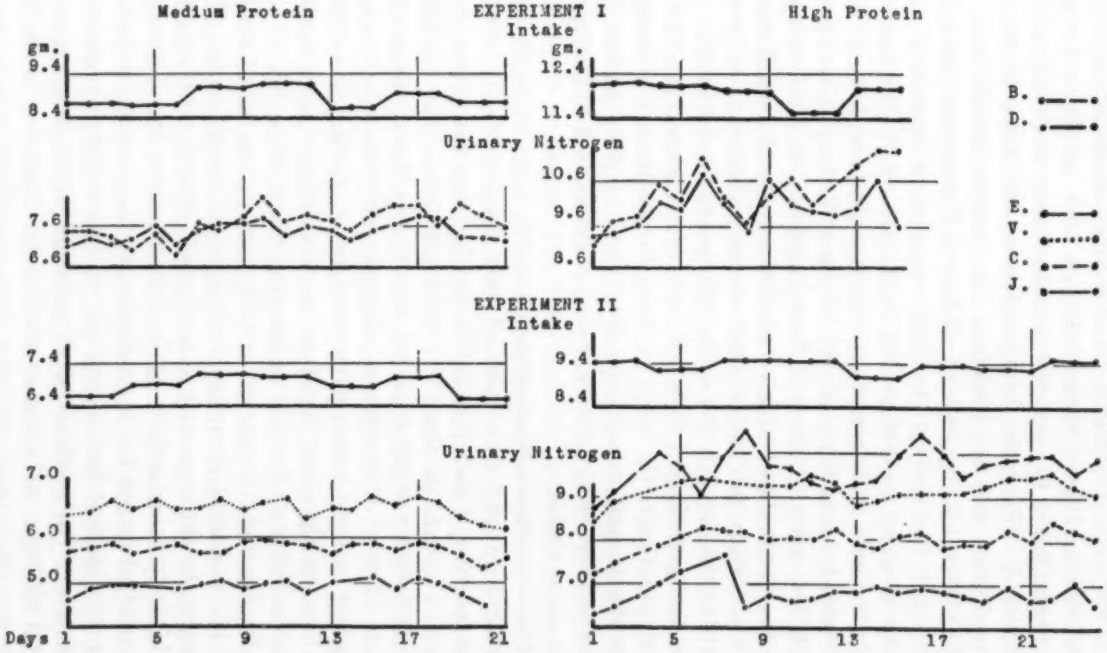


Figure 1

In the first experiment the coefficients of variation for uric acid nitrogen were about twice as high as those for total nitrogen, but in the second experiment they were only slightly increased. Since the composition of the diets in the two experiments was not identical, the changes in uric acid content of the diet may have caused the differences in variability. Although the diet in the first experiment contained about 6 gm. more of meat than that in the second, it does not seem that the uric acid from this source could have been twice as variable because the total diet nitrogen fluctuation was practically the same. Uric acid from vegetable or fruit source or from some metabolic factor must have caused the differences.

The values for amino acid nitrogen showed more irregularities than any of the other nitrogenous constituents. For the first experiment, the coefficients of variation were 11.4 and 13.8, practically the same as those for ammonia nitrogen, but for the second experiment they were slightly higher from 13.8 to 14.6. The values for each child seemed to fluctuate to the same extent.

Since creatinine is often considered to be an index of muscle metabolism and as a general rule remains fairly constant irrespective of diet, it is not surprising that the values should be as constant as those for total nitrogen. The coefficients of variation ranged from 2.5 to 3.4, and there was no more than 17 mg. of creatinine nitrogen difference between the highest and the lowest value for any one child. The creatine values, on the other hand, were considerably more variable, the coefficients of variation ranging from 4.4 to 7.6.

Pucher and his associates ('34) gave a statistical analysis of the variation in urinary nitrogenous constituents. Their results, however, cannot be compared directly with results in this study because their subjects were adults, the diet was not controlled except for the omission of meat on the days when samples were collected, and the collection days were not consecutive. The coefficients of variation which they report for total nitrogen, urea and creatinine are from five to six times greater than those reported here, but they find, as in this

study, that the values for these constituents are more constant than those for ammonia, acidity, amino acid and creatine. This fact may mean either that the technical errors in analysis of the latter constituents are greater than those for total nitrogen, urea, and creatinine or that some uncontrollable factor, not diet, influenced their excretion. The small variation in the uric acid excretion in this study may be due to the fact that the children consumed a constant amount of meat each day. If the variations which Pucher and his associates ('34) report may be considered normal for subjects living on an ordinary mixed diet, and if the coefficient of variation represented by the diet nitrogen may be considered as the minimum variation, the children in this study had certainly reached a fair degree of metabolic equilibrium, probably as great a degree as could be expected on account of the diet fluctuations.

Immediately following the change to a high protein diet, the majority of the urinary nitrogenous substances displayed more daily variation than they had on the medium protein diet. Figure 1 also shows the daily intake variation on the high protein diet as related to the total urinary nitrogen excretion of the six children. The intake values of the first experiment showed practically the same degree of irregularity as those on the medium protein diet, since the coefficient of variation was 2.3 as compared with 2.8. In the second experiment, the intake values were more constant on the high protein diet, the coefficient of variation being 1.6 instead of 3.0. Therefore, it is not surprising that the figures for total urinary nitrogen should seem to be more regular on the second than on the first experiment. Nevertheless, in all cases the total nitrogen increased rather regularly during the first 4 to 7 days. In some cases, the values became lower on the following 2 or 3 days and then they seemed to reach a more constant level. Since the figures for both intake and excretion fluctuated somewhat from day to day, it was impossible to say that the children reached an equilibrium at any one time. A 9-day period, however, apparently covered

the largest part of the irregularities and was thus considered the preliminary period.

Table 2 presents a statistical treatment of the data based on the entire 15 or 24 days of the high protein diet. It also gives the standard deviation and the coefficient of variation for the data during the first 9 days or the preliminary period for this diet, and in the second experiment, for the data on the last 15 days.

With the exception of creatinine all of the urinary nitrogenous substances increased in amount with the increase in diet nitrogen. The volume of urine remained practically the same and the daily variations were within a similar range. The figures for specific gravity averaged slightly higher, but the coefficients of variation were the same, 0.1. Total acidity increased in amount, but the coefficients of variation for each child were quite similar to those on the medium protein diet.

The change in the protein content of the diet, as indicated in figure 1, caused the total urinary nitrogen figures to be more irregular. The coefficients of variation, which ranged from 3.0 to 6.4, were slightly higher for each child, the older children having the highest values. The coefficient for E was slightly higher than the coefficients for the other children on the second experiment. His previous illness may have influenced the variability although 15 days had elapsed since he appeared normal. On the other hand, the larger fluctuation for the three older children may have been caused by their greater activity, or by a nervous tension which the younger children did not seem to have. The change in the nitrogen content of the diet must have been a factor because all other factors remained constant and the period by period variation in the diet was the same for subjects D and B and even lower for the other four children.

The alteration in the diet influenced some but not all of the other constituents. The coefficients of variation for each child for urea, ammonia and creatine were with few exceptions, higher than they had been on the medium protein diet. The values for uric acid and amino acid nitrogen showed no in-

TABLE 2

*Daily variation in 24-hour urinary excretion on a constant 4-gm. protein diet*

| FUNCTION | EXPERIMENT | CHILD | ENTIRE PERIOD | | | | FIRST 9 DAYS | | LAST 15 DAYS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Range | Mean | Standard deviation | Coefficient of variation | Standard deviation | Coefficient of variation | Standard deviation | Coefficient of variation |
| | | | | | | % | | % | | % |
| Volume (cc.) | I | D | 1009–1332 | 1156±14.3 | 82.3 | 7.1 | 69.7 | 5.9 | ... | ... |
| | | B | 1063–1344 | 1187±15.4 | 85.5 | 7.2 | 96.4 | 8.0 | ... | ... |
| | II | J | 450– 770 | 670±11.1 | 78.9 | 11.8 | 60.2 | 8.6 | 85.6 | 13.1 |
| | | C | 690– 900 | 813± 8.3 | 59.1 | 7.3 | 37.7 | 4.4 | 56.7 | 7.2 |
| | | V | 730–1080 | 946±13.5 | 89.2 | 9.4 | 111.7 | 11.3 | 78.8 | 8.5 |
| | | E | 730–1350 | 1046±24.6 | 171.3 | 16.4 | 164.5 | 14.4 | 153.4 | 15.5 |
| Specific gravity | I | D | 1.012–1.016 | 1.0142±0.0002 | 0.0012 | 0.1 | 0.0011 | 0.1 | ..... | ... |
| | | B | 1.013–1.017 | 1.0146±0.0002 | 0.0012 | 0.1 | 0.0015 | 0.1 | ..... | ... |
| | II | J | 1.010–1.015 | 1.0129±0.0002 | 0.0013 | 0.1 | 0.0016 | 0.2 | 0.0011 | 0.1 |
| | | C | 1.009–1.014 | 1.0122±0.0002 | 0.0013 | 0.1 | 0.0014 | 0.1 | 0.0008 | 0.1 |
| | | V | 1.009–1.013 | 1.0115±0.0001 | 0.0009 | 0.1 | 0.0012 | 0.1 | 0.0007 | 0.1 |
| | | E | 1.009–1.013 | 1.0113±0.0002 | 0.0011 | 0.1 | 0.0007 | 0.1 | 0.0013 | 0.1 |
| Total nitrogen (gm.) | I | D | 9.35–10.77 | 9.98±0.08 | 0.45 | 4.5 | 0.52 | 5.3 | ... | ... |
| | | B | 9.15–11.33 | 10.35±0.12 | 0.66 | 6.4 | 0.57 | 5.6 | ... | ... |
| | II | J | 6.35– 7.65 | 6.84±0.04 | 0.27 | 4.0 | 0.43 | 6.3 | 0.15 | 2.1 |
| | | C | 7.28– 8.46 | 8.04±0.04 | 0.26 | 3.3 | 0.38 | 4.8 | 0.17 | 2.1 |
| | | V | 8.40– 9.61 | 9.21±0.04 | 0.28 | 3.0 | 0.43 | 4.8 | 0.21 | 2.2 |
| | | E | 8.79–10.56 | 9.69±0.06 | 0.44 | 4.5 | 0.59 | 6.1 | 0.34 | 3.5 |
| Urea nitrogen (gm.) | I | D | 8.43– 9.82 | 8.99±0.07 | 0.42 | 4.7 | 0.51 | 5.7 | ... | ... |
| | | B | 8.11–10.02 | 9.15±0.11 | 0.60 | 6.6 | 0.52 | 5.8 | ... | ... |
| | II | J | 5.52– 6.76 | 6.15±0.04 | 0.28 | 4.5 | 0.39 | 6.6 | 0.14 | 2.3 |
| | | C | 6.44– 7.59 | 7.20±0.04 | 0.27 | 3.8 | 0.32 | 4.6 | 0.16 | 2.2 |
| | | V | 7.71– 8.87 | 8.37±0.04 | 0.27 | 3.3 | 0.31 | 3.8 | 0.23 | 2.8 |
| | | E | 7.91– 9.60 | 8.75±0.06 | 0.41 | 4.7 | 0.43 | 5.1 | 0.33 | 3.7 |
| Ammonia nitrogen (mg.) | I | D | 220–321 | 293±4.5 | 25.6 | 8.7 | 30.8 | 10.4 | ... | ... |
| | | B | 360–527 | 428±8.8 | 48.7 | 11.4 | 56.9 | 12.9 | ... | ... |
| | II | J | 109–199 | 142±3.1 | 22.0 | 15.5 | 26.5 | 18.4 | 20.2 | 14.2 |
| | | C | 185–279 | 217±3.5 | 24.6 | 11.3 | 30.3 | 13.8 | 22.2 | 10.2 |
| | | V | 172–276 | 221±3.8 | 25.5 | 11.5 | 28.8 | 12.6 | 24.9 | 11.4 |
| | | E | 171–285 | 233±4.6 | 31.9 | 13.7 | 35.8 | 15.4 | 30.8 | 13.2 |
| Acidity (cc. 0.1 N acid) | I | D | 202–265 | 236±3.2 | 18.2 | 7.7 | 19.3 | 8.0 | ... | ... |
| | | B | 254–334 | 295±4.2 | 23.5 | 8.0 | 25.9 | 8.7 | ... | ... |
| | II | J | 76–123 | 94±1.6 | 11.5 | 12.2 | 11.2 | 12.1 | 12.0 | 12.7 |
| | | C | 74–149 | 97±2.5 | 17.8 | 18.4 | 8.5 | 9.1 | 21.2 | 21.5 |
| | | V | 90–139 | 115±2.2 | 14.5 | 12.7 | 15.3 | 13.0 | 14.7 | 12.9 |
| | | E | 118–215 | 162±3.2 | 22.3 | 13.8 | 27.5 | 17.7 | 18.7 | 11.3 |
| Uric acid nitrogen (mg.) | I | D | 97–113 | 105±0.8 | 4.5 | 4.3 | 4.5 | 4.3 | ... | ... |
| | | B | 119–161 | 138±2.0 | 11.2 | 8.1 | 8.6 | 6.5 | ... | ... |
| | II | J | 73– 84 | 79±0.4 | 2.8 | 3.6 | 2.9 | 3.8 | 2.7 | 3.4 |
| | | C | 95–111 | 102±0.6 | 4.4 | 4.3 | 2.0 | 2.0 | 3.9 | 3.7 |
| | | V | 108–125 | 116±0.7 | 4.9 | 4.3 | 6.7 | 5.9 | 4.1 | 3.5 |
| | | E | 92–109 | 100±0.7 | 4.6 | 4.7 | 3.9 | 4.1 | 4.0 | 3.9 |
| Amino acid nitrogen (mg.) | I | D | 80–100 | 90±1.1 | 6.1 | 6.8 | 4.9 | 5.7 | ... | ... |
| | | B | 83–120 | 96±1.9 | 10.4 | 10.8 | 5.2 | 5.7 | ... | ... |
| | II | J | 99–160 | 139±2.6 | 18.5 | 13.3 | 18.4 | 15.3 | 7.0 | 4.7 |
| | | C | 123–204 | 177±3.4 | 24.4 | 13.8 | 20.2 | 13.6 | 8.7 | 4.6 |
| | | V | 132–222 | 193±3.9 | 25.9 | 13.4 | 30.3 | 18.8 | 11.8 | 5.8 |
| | | E | 148–257 | 207±4.4 | 30.6 | 14.8 | 21.6 | 12.4 | 15.9 | 7.1 |
| Creatinine nitrogen (mg.) | I | D | 133–166 | 150±2.2 | 12.5 | 8.3 | 10.2 | 6.5 | ... | ... |
| | | B | 148–170 | 156±1.2 | 6.5 | 4.2 | 6.6 | 4.2 | ... | ... |
| | II | J | 71– 78 | 74±0.2 | 1.7 | 2.3 | 1.6 | 2.1 | 1.7 | 2.3 |
| | | C | 82– 90 | 86±0.3 | 2.4 | 2.8 | 2.1 | 2.4 | 2.6 | 3.1 |
| | | V | 100–112 | 106±0.6 | 3.8 | 3.6 | 1.7 | 1.5 | 2.5 | 2.4 |
| | | E | 118–126 | 122±0.3 | 2.4 | 2.0 | 2.9 | 2.4 | 2.2 | 1.8 |
| Creatine nitrogen (mg.) | I | D | 130–189 | 155±2.8 | 15.4 | 10.0 | 20.4 | 13.1 | ... | ... |
| | | B | 160–211 | 188±2.5 | 14.1 | 7.5 | 14.1 | 7.7 | ... | ... |
| | II | J | 74–102 | 90±1.2 | 8.8 | 9.9 | 6.2 | 6.6 | 9.5 | 10.8 |
| | | C | 78–108 | 92±1.1 | 8.1 | 8.7 | 9.3 | 9.6 | 6.5 | 7.2 |
| | | V | 97–122 | 108±1.0 | 6.5 | 6.0 | 9.2 | 8.2 | 4.9 | 4.6 |
| | | E | 100–152 | 121±2.2 | 15.6 | 12.9 | 14.7 | 10.8 | 7.8 | 7.0 |

crease in variability and those for creatinine remained just as constant as they had been on the medium protein diet.

During the first 9 days of the high protein diet, those constituents which were apparently most influenced by diet, usually showed a greater irregularity than they did for the entire period. The coefficients of variation for total nitrogen, urea and ammonia were higher for every child, except B, and those for creatine increased for four of the six children. The other constituents showed no constant change.

In the second experiment, there was less variation for some of the constituents during the last 15 days of the high protein diet than there had been at any time during the entire study. The coefficients of variation for total nitrogen and urea were lower than they had been on the medium protein diet. The values for ammonia, acidity and uric acid were no more constant than they were when all of the data on the high protein diet were considered. The figures for amino acid nitrogen were more constant than they had been at any other time. With the exception of the figures for J, the creatine values were less variable than they had been for the total high protein diet, but not as constant as they were on the medium protein diet. Creatinine figures seemed to be exceedingly constant throughout the entire study and were apparently affected little by the change in diet. The variability in the values for the two children in the first experiment and for V was slightly greater on the medium protein diet.

The data for total nitrogen on the last 15 days of the high protein diet were just as constant as that on the medium protein diet. Thus it can be assumed that, if the children were in nitrogen equilibrium during the medium protein diet, they were probably in equilibrium during the last 15 days of the high protein diet and that the 9-day preliminary period was sufficiently long for this study. Greater changes in diet might require a longer preliminary period.

The individual differences among the children were in reality quite small. In general, the coefficient of variation for one particular constituent was almost the same for each

child. The increase in variability following the change in diet was again similar. Therefore, the data seem to indicate that under identical conditions the children reacted the same.

So far the discussion has dealt entirely with the variability of the data obtained on individual children and not with the variability in the data as a whole. In order to combine the figures for several children it was necessary to use the values expressed in terms of a common measurement, milligrams per kilogram of body weight. Table 3 gives the coefficients of variation obtained from the statistical treatment of the data per kilogram on each experiment and for both experiments together, for the medium and high protein diets and also for the first 9 days of the high protein diet, and for the last 15 days of the high protein diet on the second experiment.

The combination of the intake values per kilogram for several children produced coefficients of variation which were somewhat different from those for the data of individual children. For the first experiment the values were similar, but for the second and for both experiments considered together the coefficients were increased. In all cases the figures were slightly more variable on the medium than on the high protein diet. It is probable that several factors beside the variation in the composition of identical diets produced this variability, because, even though the children received food in amounts proportional to their body weight. the amount of nitrogen consumed varied slightly from child to child. In the first place, it was impossible to get an exact proportion of each food without using small fractions of a gram which would have been impractical. Secondly, the weights of the children changed somewhat during the experiment. Finally, there were greater differences between the nitrogen intakes of the children on the two experiments than there were between different children on the same experiment.

In general, the coefficients of variation for all of the nitrogenous excretory products were higher than they were for individual children. Nevertheless, the data for total nitrogen and urea on each experiment showed the same tendencies as

they did for individual children. In spite of the lower variation in intake on the high protein diet, the coefficients of variation increased following the change in diet. The coefficients were highest during the first 9 days on the high protein diet and similar to those on the medium protein diet on the last 15 days of the experiment.

TABLE 3

*Coefficients of variation of the per kilogram intake and urinary output*

| FUNCTION | EXPERIMENT | MEDIUM PROTEIN DIET | HIGH PROTEIN DIET | | |
|---|---|---|---|---|---|
| | | | All | First 9 days | Last 15 days |
| | | % | % | % | % |
| Intake | I | 2.3 | 2.0 | 1.0 | |
| | II | 3.8 | 3.2 | 3.0 | 3.3 |
| | Both | 6.4 | 5.5 | 5.8 | |
| Total urinary nitrogen | I | 4.7 | 5.9 | 6.5 | |
| | II | 4.8 | 5.2 | 6.0 | 4.8 |
| | Both | 6.1 | 5.9 | 6.6 | |
| Urea nitrogen | I | 5.6 | 6.5 | 7.4 | |
| | II | 4.3 | 5.5 | 6.0 | 4.7 |
| | Both | 7.1 | 6.1 | 7.2 | |
| Ammonia nitrogen | I | 18.7 | 18.2 | 20.0 | |
| | II | 15.1 | 17.9 | 19.4 | 17.3 |
| | Both | 28.5 | 27.7 | 30.4 | |
| Uric acid nitrogen | I | 10.3 | 11.4 | 9.7 | |
| | II | 9.1 | 11.5 | 11.3 | 11.3 |
| | Both | 11.3 | 11.7 | 11.1 | |
| Amino acid nitrogen | I | 12.3 | 8.8 | 6.1 | |
| | II | 15.3 | 15.0 | 14.8 | 8.7 |
| | Both | 25.5 | 31.1 | 32.5 | |
| Creatinine nitrogen | I | 3.4 | 7.1 | 6.7 | |
| | II | 7.1 | 8.6 | 8.6 | 8.5 |
| | Both | 12.9 | 17.7 | 19.4 | |
| Creatine nitrogen | I | 6.1 | 10.0 | 10.7 | |
| | II | 7.7 | 10.0 | 10.0 | 7.7 |
| | Both | 7.7 | 20.5 | 18.4 | |
| Acidity | I | 10.9 | 10.5 | 10.4 | |
| | II | 13.7 | 19.3 | 18.1 | 20.0 |
| | Both | 34.6 | 38.2 | 39.5 | |
| Volume | I | 11.3 | 7.7 | 7.7 | |
| | II | 9.7 | 13.3 | 12.7 | 12.5 |
| | Both | 10.8 | 14.2 | 12.6 | |

The collective data for total nitrogen and urea in the two experiments showed the highest coefficients of variation on the first 9 days of the high protein diet, but the variation in all of the data for the high protein was less than that for the medium protein diet. Nevertheless, a comparison of the coefficients of variation for intake and total nitrogen showed that the values on the medium protein diet decreased from 6.4 to 6.1 and those on the high protein diet increased from 5.5 to 5.9. Therefore, the collective values probably show the same tendencies as do the individual figures, but the differences between the children and between the two experiments partially mask those tendencies.

The data for ammonia and creatine are less conclusive. In the second experiment the figures display the same tendencies as total nitrogen, but on the other experiment and on the collective data the relationship was not clearly portrayed.

The values for uric acid, amino acid, acidity and volume showed approximately the same variability on both diets. Thus the collective figures, as well as those for individual children, indicated that these constituents were not influenced by diet. The figures for creatinine became more variable after the change to the high protein diet, but they were not more irregular on the first 9 days. Thus, as indicated by the data on individual children, the diet had less influence on creatinine than on total nitrogen.

The results from the collective data indicate that individual tendencies may be obviated by combining the data because the collective data show more variation than that for one experiment or that for an individual child. The collective data also point out the danger of combining figures from experiments which were not identical. Although the two experiments in this study were practically the same, the differences were great enough to influence the variability of the results.

SUMMARY

1. The amount of total urinary nitrogen, urea, creatinine and, in one experiment, uric acid excreted by six children on constant medium protein diets following a 10- or 12-day preliminary period varied to approximately the same degree as the values for diet nitrogen.

2. Uric acid in one experiment and creatine in both were about twice as variable as diet nitrogen, while ammonia, acidity and amino acid showed more irregularity.

3. The increase in the protein content of the diet caused the values for total nitrogen, urea, ammonia and creatine to be more variable, especially during the first 9 days. Then the figures reached an equilibrium similar to that on the first diet. Therefore, in this study a 9-day preliminary period seemed to be adequate.

4. The change in diet did not seem to influence the variability of the data for acidity, uric acid, amino acid or creatinine.

5. Individual children tended to react in a similar manner both to the constant diet and to the change in the protein content of the diet.

6. When the values were expressed on the basis of kilograms of body weight, the coefficients of variation of the data became larger in all cases. The values for either experiment showed the same tendencies as the data for individuals, but the collective values for the two experiments tended to obscure the fluctuations.

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# A RESPIRATION CHAMBER FOR USE WITH HUMAN SUBJECTS[1]

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THREE FIGURES

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The respiration chamber devised by F. G. Benedict et al. ('34) for large animals has been modified by us for the study of the human subject. It was constructed to measure the total transformation of energy (heat production) during 24 hours. It may also be used for longer or shorter periods. The shortness of the period is necessarily determined by the time required for complete mixing of the gases. For example, about 80 liters of $CO_2$ were rapidly introduced into the closed chamber in which the mixing pump was running. The inlet was then stoppered. Samples withdrawn 30 minutes later gave a value of 0.816% $CO_2$. After another 30 minutes, samples yielded a value of 0.815%. Therefore complete mixing had occurred by the end of the first ½ hour. The apparatus employs the principle of open circuit indirect calorimetry.[2]

The subject is enclosed in a sealed space from which air is removed at a constant rate by means of a pump. Spirometers continuously sample the air leaving the chamber so that they contain a true aliquot of all the outgoing air. This aliquot is analyzed for its percentile composition of carbon dioxide and oxygen. We employ the apparatus devised for this pur-

[1] This study was assisted by a special grant to L. H. Newburgh from the Horace H. Rackham Endowment Fund.

[2] An extended discussion of this method has been presented by E. F. DuBois ('36).

pose by T. M. Carpenter ('33). As pointed out by Carpenter we were able to make duplicate analyses which checked within 0.004% for $CO_2$ and 0.005% for $O_2$. The apparatus was frequently checked by analyses of outdoor air. The total volume of outgoing air is recorded by calibrated gas meters. The incoming air is outdoor air which has a fixed percentile composition of carbon dioxide and oxygen. When the composition and the volume of the outgoing air and the composition of the incoming air are known, one may calculate the absorption of oxygen and production of carbon dioxide. To calculate the heat production one must also determine the urinary nitrogen. With these data, the calculation proceeds in the standard manner.

The chamber (fig. 1) consists of an inverted tank or hood hung on four steel cables that pass over pulleys near the ceiling of the laboratory to counterpoises whose combined weight is roughly equal to that of the hood. The upper and lower corners of the hood are supplied with rollers that fit into channels which are fastened to the floor and ceiling of the laboratory. These channels fix the position of the hood and guide its upward and downward movement. When the hood is pulled down the lowest 2 inches of its walls descend into oil[3] contained in a trough which is sealed into the concrete floor.

The spirometers (fig. 2) are of the type used to measure vital capacity.[4] However, the tube through which the gas passes into the sealed space is only $\frac{1}{16}$ inch inside diameter. The volume of the bell is about 7 liters. The counterpoise is specially designed to move in the sleeve with the least possible friction, and carries a pointer that moves past a graduated bar. The bell is attached to the weight by a chain that passes over a pulley whose axle and the seat in which the axle rides are carefully milled to reduce friction. Oil[3] is used as a seal. Since the weight of the bell will increase relative to the weight of the counterpoise as the former moves up out of the oil,

[3] Squibb's mineral oil.

[4] Obtained from Warren E. Collins, Inc., 555 Huntington Ave., Boston.

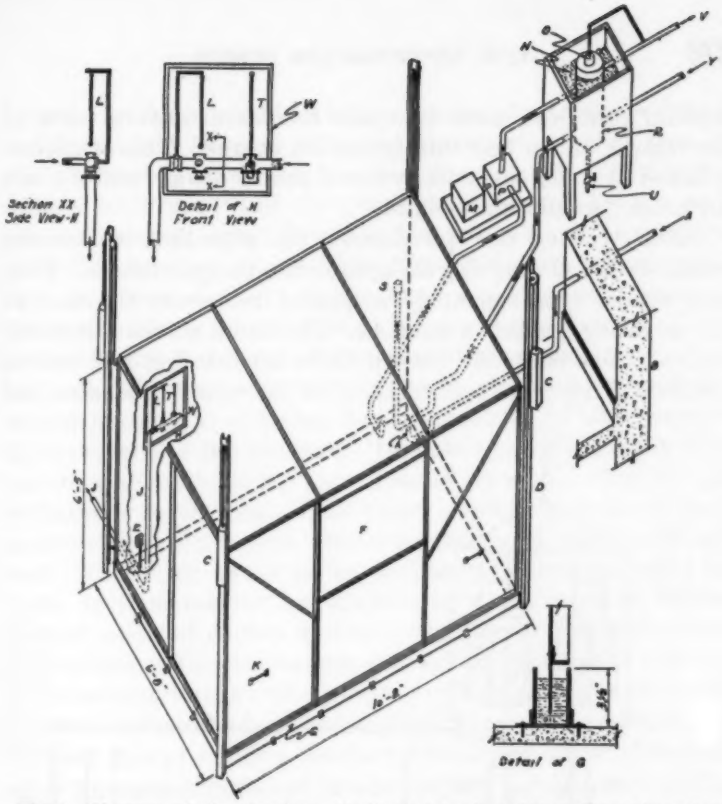


Fig. 1 Diagram of the chamber. A, duct through which outdoor air enters the chamber. B, outer wall of laboratory. C, lead weights, attached to the upper corners of the chamber by means of flexible steel cables that pass over pulleys in the top of the channels, D. The upper and lower corners of the chamber are supplied with rollers that move in the channels. D, the channels are fastened to the floor and ceiling of the laboratory. E, electric outlet. F, plate glass, sealed into wall of chamber. G, steel trough containing mineral oil. T, wet bulb thermometer in the upper end of the duct, J, through which the air leaves the chamber. H, the wick of the wet bulb thermometer dips into a wide mouthed bottle containing distilled water and held in position by rubber bands. The position of the valve causes the air to pass by the wet bulb or the wet bulb may be excluded from the circuit. A loose ball of copper wire soaked in oil, in each of the inlets to duct J, filters the air. K, handles.

The assembly whose parts are marked with the letters M through Y is a device for lowering humidity and cooling the air within the chamber. M and P, motor and pump. Cold brine enters a coil suspended inside of the sheet brass cylinder, R, and leaves through V. The rate of flow of the brine is regulated through the thermostatic valve, O. The cylinder is embedded in granulated cork, N. Water that condenses in the cylinder is removed at the end of an experiment by means of valve U. W, window in wall of chamber to permit observation of the wet bulb thermometer, T, and the dry bulb thermometer, L.

special provision must be made to insure uniform rate of movement of the bell throughout its course. This is accomplished by using a counterpoise of proper weight and a chain of proper weight and length.

After passing the spirometers, the pipe that carries the main stream of outgoing air approaches the gas meters. They are of the type commonly employed to record the flow of illuminating gas into a building. The hands on the last meter move at one-tenth the rate of those on the first and second meters. They were calibrated, after the whole apparatus had

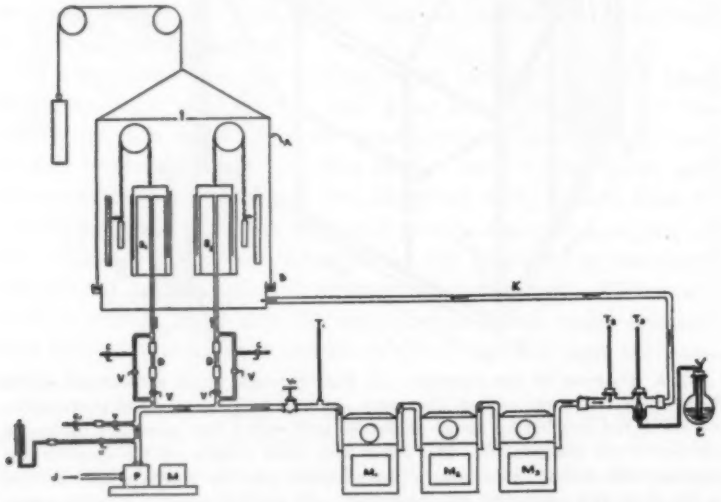

Fig. 2 Spirometers and gas meters. A, hood which when closed causes the spirometers to be surrounded by chamber air. A hole in the top of the hood allows escape of air. B, trough for water seal. $S_1$, $S_2$, spirometers whose bells are hung on chains that pass over pulleys to counterpoises. C, thick walled rubber tubing and clamp, through which samples from the spirometers are withdrawn. $C_1$, similar device for obtaining samples of outgoing air. D, glass capillary tubes. The rate of sampling is regulated by the internal diameter of these tubes. V, one-way brass valves. $V_1$, globe valve. G, mercury manometer. J, continuation of duct through which the air leaves the chamber. P, pump driven by electric motor M. $M_1$, $M_2$, $M_3$, gas meters. $T_1$ and $T_2$, dry bulb and $T_3$, wet bulb, thermometers. E, flask containing distilled water. K, duct that leads the outgoing air into the box that encloses the spirometers.

been set up by connecting a large cylinder of nitrogen weighed to 0.1 gm., to a Douglas bag and then to the outgoing pipe of the chamber. After the bag had been partly inflated the meters were read and the pump turned on. As the bag emptied more gas was allowed to flow into it from the cylinder until the latter was empty. The instant the bag had emptied, the pump was stopped and the meters read again. Factors for each meter were obtained in the usual manner.

Beyond the last meter, the stream of air passes across dry and wet bulb thermometers and thence into the bottom of the compartment that contains the spirometers. This compartment consists of two sections. The lower one is a shallow box carrying a trough on the outer face of its walls. The trough contains a water seal. The upper section is a hood that encloses the spirometers. Its lower edges fit into the water seal. The outgoing air is led back into the compartment in order to immerse the spirometers in a gas mixture whose concentration of carbon dioxide is similar to that within the spirometers. The air escapes through an opening in the top of the compartment. The purpose of this arrangement is to prevent diffusion of carbon dioxide from the spirometers. Without this precaution, we were first able to detect diffusion when the concentration of carbon dioxide had reached 0.45%.

Diffusion from the chamber itself must also be considered. In order to deal with this question we allowed $CO_2$ to flow into the chamber through the sampling duct and then closed the inlet pipe. The gas within the chamber was mixed as usual. When the concentration of $CO_2$ within the space was 0.475%, no change was detectable 20 hours later. However, when the initial concentration was 0.616%, it fell to 0.606% in 21 hours. An initial concentration of 0.816% was reduced to 0.801% in 22 hours. Expressed in volumes, these data show that 1 liter of $CO_2$ was lost when the initial concentration was 0.616%; and that 1.5 liters of $CO_2$ were lost when the initial concentration was 0.816%. Accordingly it is advisable to prevent the concentration of $CO_2$ in the chamber from rising above 0.5% by proper regulation of the ventilation.

However, when the concentration of $CO_2$ is 0.6% for not more than 4 hours, the concentration will fall only 0.002%—a negligible change.

In order to make the chamber independent of the weather, the air is cooled and dried by refrigeration. A ½ inch pipe is brought out of the chamber without breaking the seal and is attached to an air pump. The latter delivers the air into the top of a closed sheet copper cylinder. Several inches above the bottom of the copper box a second pipe leads the air back into the respiration chamber. Running through the copper container is a cold brine line supplied with fins. The rate of flow of the brine is controlled by an automatic valve activated by the temperature of the air. The bottom of the copper vessel is furnished with a valve by means of which water may be drained away at the end of an experiment.

The chamber contains a standard single bed, a chair, a table, a reading lamp, telephone and radio connection. The subject is supplied with food of the desired composition contained in air tight vessels. The urine is voided into enameled cans whose lids are designed to minimize evaporation. The stool is passed into large tin cans (diameter 8 inches) whose lids fit so tightly that the annoyance of prolonged fecal odor within the chamber is done away with. A commode is on hand for subjects who desire to use it.

### THE VOLUME OF THE CHAMBER

The inside dimensions of the closed chamber were measured and the volume calculated. They are: length, 3.043 M; width, 1.992 M and height, 1.922 M. The calculated volume is 11,650 liters. This volume is decreased somewhat by the necessary furnishings of the chamber and by the subject who is in it. On the other hand, it is augmented by the pipes leaving the chamber, the blower and the meters. Consequently we have used the value of 11,600 liters as the working volume. This must be close to the true volume and if it were wrong by 100 liters the error involved in the calculation of the respiratory exchange would be less than the error of the whole method.

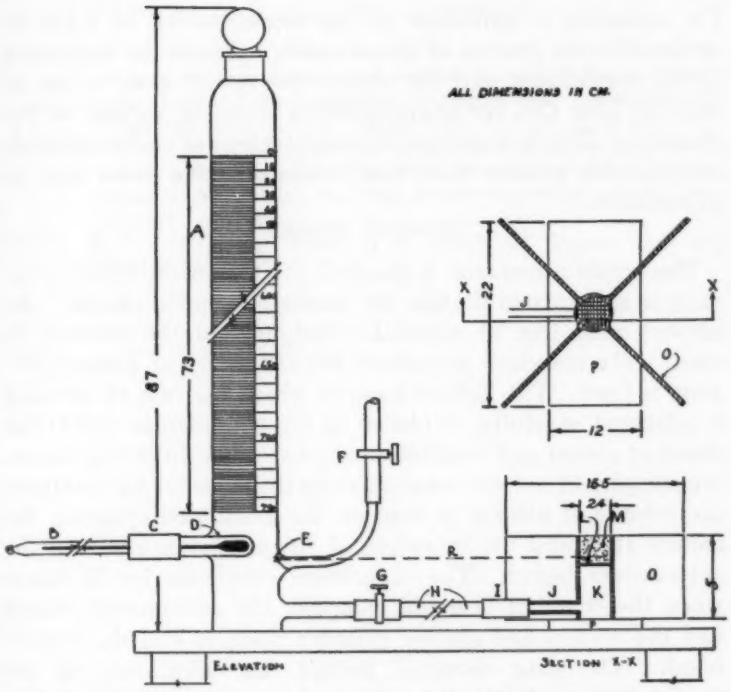


Fig. 3 Alcohol burner. A, cylinder graduated to 2 mls. and closed by a ground glass stopper (air tight). B, thermometer held in thermometer seal, D, by the rubber tubing, C. E, capillary inlet for air. The alcohol in the burner seeks the level of the air inlet. This capillary inlet keeps the bubbles small so that there is no interference with the volume readings. Openings, E and H, are closed by clamps, F and G, while filling cylinder. The outflow tube, H, connects cylinder with burner by rubber tubing, I, to copper tube, J. K, chamber in which alcohol collects and rises to level, R. L, screen on which shredded asbestos, M, rests. O, copper fins to carry away the heat of the flame. P, base of burner.

To regulate the flame, the apparatus is set up so that the level of alcohol in the burner is between the screen and the top of the burner. Shredded asbestos, the highest part of which is above the original level of the alcohol, is placed loosely in the burner. It is then lighted and when the alcohol burns evenly, the level of alcohol in the feeding cylinder, A, and the time are recorded. Then after a period of 10 minutes, for example, another reading is taken and the milliliters per hour calculated. By changing the level of the asbestos the flame can be adjusted to consume alcohol to within 1 or 2 ml. per hour of the desired rate.

For example, a difference in the concentration of 0.1% in carbon dioxide content of the chamber between the beginning of the experiment and the end would result in an error of only 0.1 liter $CO_2$ for every 100-liter error in volume of the chamber. This is a change in concentration of carbon dioxide considerably greater than that obtained in the usual type of experiment.

### ALCOHOL CHECKS

The whole apparatus is checked for tightness, recovery of oxygen and carbon dioxide by means of alcohol checks. An alcohol lamp (fig. 3) especially designed for the chamber is used. The standard procedure for the study of human subjects is used. The lighted lamp in which the rate of burning is adjusted carefully, is placed in the chamber at night; the chamber closed and ventilation started. The following morning samples of air are removed from the chamber for analysis, the volume of alcohol is read on the graduated cylinder, the meters read and the collection of the aliquot samples in the spirometers begun. The experiment continues for 24 hours when the chamber is again sampled, the spirometers closed and the meters and alcohol cylinder read, to end the experiment. The data obtained permit the calculation of the respiratory quotient, the oxygen absorption and the carbon dioxide production.

A series of checks is shown in table 1.

TABLE 1

*Alcohol checks*

| DATE | ALCOHOL BURNED | R.Q. | CARBON DIOXIDE | | | OXYGEN | | |
|---|---|---|---|---|---|---|---|---|
| | | | Theoretical | Determined | Recovery | Theoretical | Determined | Recovery |
| | *gm.* | | *liters* | *liters* | % | *liters* | *liters* | % |
| 7– 5–35 | 260.9 | 0.668 | 254.0 | 253.7 | 99.9 | 281.1 | 279.9 | 99.7 |
| 9–17–35 | 369.0 | 0.682 | 359.4 | 357.1 | 99.4 | 539.2 | 523.8 | 97.1 |
| 9–30–35 | 428.3 | 0.662 | 417.0 | 412.6 | 98.9 | 625.5 | 623.0 | 99.6 |
| 4– 8–36 | 297.3 | 0.661 | 289.1 | 287.5 | 99.4 | 433.7 | 434.3 | 100.1 |
| 6–25–36 | 335.3 | 0.660 | 326.4 | 319.3 | 97.8 | 489.7 | 483.5 | 98.7 |
| Average | | 0.667 | | | 99.1 | | | 99.0 |

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# FURTHER EXPERIENCES WITH THE MEASUREMENT OF HEAT PRODUCTION FROM INSENSIBLE LOSS OF WEIGHT [1]

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It is well known that vaporization of water is one of the mechanisms by means of which the body rids itself of heat. This portion of the outgoing heat is removed at the rate of 0.58 of a large calorie per gram of water vapor.

It has been suggested that a quantitative relationship exists between the total heat elimination and that portion which is removed by the vaporization of water. We have been studying this relationship and now propose to deal specifically with the following questions: 1) Can an accurate procedure be devised for the calculation of water vapor? 2) Does the organism always lose a fixed percentage of the heat of its metabolism by vaporization of water, and if not, are there conditions when it will do so?

The direct measurement of water vapor is very difficult. However, since the water vapor always accounts for the greater part or all of the insensible loss of weight, the possibility of calculating it from the latter exists.

The insensible loss of weight is easily obtained by substitution in the following equation:

I.L. = (Initial Body Weight + Ingesta)—(Final Body Weight + Urine + Stool),

where I.L. is insensible loss of weight.

The composition of the insensible loss of weight is accurately defined in the following equation:

$$I.L. = I.W. + CO_2 - O_2,$$

[1] This study was assisted by a special grant to L. H. Newburgh from the Horace H. Rackham Endowment Fund.

where I.W. is the weight of the water vapor or insensible water; and $CO_2$ and $O_2$ are the weights of the two respiratory gases. It must be understood that insensible water means only that water which leaves the body in the form of vapor. The water vapor is always the largest part of the insensible loss of weight and may be the whole of it, as already noted. When the organism is metabolizing a mixture that necessitates an absorption of oxygen whose weight equals that of the carbon dioxide produced,

$$\text{I.W.} = \text{I.L.}$$

This same fact may be expressed in terms of the respiratory quotient. Thus when the weights of the two gases are equal, their volume relationships are such that the respiratory quotient is 0.725. As is generally understood, such a metabolic mixture is one in which a very small proportion of the calories are yielded by the oxidation of carbohydrate. As the carbohydrate of the metabolic mixture increases, the weight of the carbon dioxide becomes proportionately greater than that of the oxygen. When the mixture, for example, is such that the respiratory quotient is 0.90, the organism is producing 0.9 liter of carbon dioxide for every liter of oxygen it absorbs. The corresponding weights of the two gases are then 1.769 gm. and 1.429 gm., and the difference is 0.34 gm. for every liter of oxygen absorbed.

In order to derive the weight of the water vapor from the insensible loss of weight one must, therefore, determine the weights of the carbon dioxide and oxygen for the period.

We have already published several papers dealing with these questions. In the first one (Wiley and Newburgh, '31) it was shown that a naked subject in the post-absorptive state, lying quietly upon steel ribbons, lost a different percentage of the heat of his metabolism through vaporization of water with every change in the temperature of the air surrounding him. When, however, he clothed himself in such a way that he felt neither uncomfortably cool nor warm, he exhibited a striking tendency to lose a fixed percentage of the heat by

evaporation of water, over a considerable range of environmental temperatures.

Subsequently, we determined what percentage of the total heat was removed by vaporization during consecutive 24-hour periods by a group of men and women who continued to lead their usual lives (Newburgh, Wiley and Lashmet, '31). They were asked to adjust their clothing to avoid conscious discomfort from heat and cold. The experiments were conducted during the winter months. The subjects spent most of their waking hours in buildings heated to the usual temperature, and each of them was out-of-doors several times each day for a few minutes at a time. They all slept with the windows of the bedroom open. They were asked to avoid strenuous exercise such as hand ball. Two of the subjects were diabetics. The remainder of the group were normal young men and women busily occupied with the care of patients or with work in the laboratory. The nude, fasting weight of each individual was recorded each morning at the same time on a balance[2] sensitive to 1 gm. The prescribed diet was prepared and weighed in the special diet kitchen of the hospital. The foods used varied from day to day. The weights of the urine and stools were determined each 24 hours.

The study was based on the following conceptions: A well-nourished adult who is following a routine of life and who is fed a fixed diet, the calorific value of which approaches his maintenance requirement, will shortly establish nitrogen balance. In addition, the glycogen store in the liver will come into balance with the carbohydrate of the diet. Thereafter, the individual will oxidize carbohydrate in the amount supplied by the diet provided the time interval under consideration is not too brief. It was not expected that this principle would hold for each cycle of 24 hours, but that the subject would be in carbohydrate balance if each period lasted 1 week. Under these circumstances, change in body weight could be attributed to deposition or loss of either adipose tissue or water. Since there was no reason for suspecting a disturbance of water metabolism in regard to any of the individuals,

[2] Obtained from Henry Troemner, Philadelphia.

change in body weight was attributable to adipose tissue provided the observations of any subject were continued for several weeks. When the record was completed, the average daily metabolic mixture corresponded with the diet except that any change in body weight over the whole period had to be reduced to a daily average, and then 90% of it either added to or subtracted from the dietary fat.[3] The calories of this metabolic mixture were taken to be the daily heat production of the individual The total change of weight of three of the subjects (table 1, T.M., M.W., M.P.) was insignificant. In those instances, heat production equalled the calories of the diet.

Having arrived at the metabolic mixture, the production of carbon dioxide and the absorption of oxygen could be calculated. Since the insensible loss of weight had been determined for each 24 hours, the weight of the insensible water could be derived by means of the equation

$$\text{I.W.} = \text{I.L.} - (CO_2 - O_2).$$

When I.W. is known, the heat removed by it is simply I.W. $\times$ 0.58. From this value and the heat production, the percentage of heat removed by the vaporization of water was calculated by means of the expression

$$\frac{\text{I.W.} \times 0.58}{\text{Heat Production}}$$

In order to arrive at a diet that would suit our purpose we fed what experience led us to believe was a maintenance diet, measured the insensible loss of weight daily for at least 1 week, calculated the gaseous exchange for that diet, derived the I.W. and assumed that 24% of the heat was removed by vaporization. The final diet was then constructed to contain the number of calories indicated by this approximation to the individual's heat production. The distribution of the various foodstuffs was the usual one for this part of the world.

[3] Since adipose tissue contains 10% of water and dietary fat is expressed in anhydrous terms, 90% of the change in weight was used.

However, in the case of the two diabetic subjects, it was not necessary to use a circuitous method to arrive at the materials oxidized since both had been living on carefully prescribed, weighed diets for years; and had long since come into balance.

In two instances, the available energy of the diet was determined by combusting a sample in the bomb calorimeter; and by subtracting from the value thus obtained, the residual heat in the urine and feces, also measured by means of the

TABLE 1

*Average percentile loss of heat by vaporization of water*

| SUBJECT | PERIOD | AGE | SEX | HEAT LOST BY VAPORIZATION OF WATER | REMARKS |
|---|---|---|---|---|---|
| First group | | | | | |
| | *days* | *years* | | % | |
| T.M. | 72 | 56 | M. | 23.8 | Diabetic |
| F.D.J. | 5 | 30 | M. | 24.2 | Diabetic |
| F.H.W. | 18 | 28 | M. | 24.2 | Chemist |
| M.W. | 44 | 24 | F. | 24.4 | Chemist |
| M.P. | 35 | 25 | M. | 24.1 | Medical student |
| A.W. | 68 | 23 | M. | 24.7 | Graduate student |
| R.L.G. | 72 | 24 | M. | 25.2 | Graduate student |
| Second group | | | | | |
| B.DeV. | 145 | 24 | M. | 24.2 | Student |
| H.J. | 30 | 18 | M. | 24.3 | Patient in bed |
| S. | 30 | 15 | M. | 24.8 | Patient in bed |
| P. | 30 | 18 | M. | 24.8 | Patient in bed |
| W.B. | 25 | 47 | M. | 24.7 | Patient in bed |

calorimeter. These checks were in good agreement with the calorific values of the diets calculated from the food tables.

Table 1 shows the average percentage of the total heat lost through evaporation of water by each subject; but it gives no information about the extent of daily variation from the average.

This first group of seven adult persons whose lives were restricted only in the few important ways noted above, showed a striking tendency to rid themselves of the same proportion of the heat of their metabolism by vaporization of water.

The average value for the group was 24.4%. The individual variation was from 23.8% to 25.2%.

Subsequently a second group of individuals was studied (table 1). To obtain greater accuracy, the nitrogen exchange was actually determined and the diets were restricted to a few foods whose composition showed little tendency to vary. They were milk, 40% cream, butter, bread, cheese, eggs, jelly and canned grapefruit. The cheese was obtained in 5-pound bricks and samples were analyzed for nitrogen and water at the beginning and end. The diets were prepared in our laboratory and all the weighings were made by the investigators.

The data from one of this second group of subjects are reported in some detail.

B. DeV. was a male, 24 years of age, 5 feet, 11 inches tall. He was a student in the school of architecture. He occupied a room set aside for the purpose in the laboratory. He walked 4 to 6 miles each day in the open to classes. He ate the diet for 3 weeks before the study began.

| | gm. |
|---|---|
| Body weight October 9, 1933 | 60,108 |
| Body weight March 3, 1934 | 60,900 |
| Total gain | 792 |
| Gain per day | 5.46 |
| Dietary nitrogen, average per day | 9.01 |
| Excretory nitrogen, average per day | 8.91 |
| Balance | +0.10 |

Since he was in nitrogen balance, the added body weight was assumed to be adipose tissue and since approximately 10% of the latter is water, it was assumed that he stored 4.91 gm. of fat daily containing 45.7 Calories ($4.91 \times 9.3$).

He ingested 2750 Calories and stored 45.7 Calories, therefore, he produced 2704 Calories. Since the diet was eaten just after being taken from a refrigerator it was cold. We calculated that it required 40 Calories to bring it up to body temperature. The dissipation of heat was accordingly 2664 Calories ($2704 - 40$). The diet had the following composition: 9.01 gm. nitrogen; 156 gm. fat; 279 gm. carbohydrate.

Since he was in nitrogen and carbohydrate balance and had stored 4.9 gm. fat daily, the metabolic mixture was 9.01 gm. nitrogen, 151 gm. fat and 279 gm. carbohydrate. The difference between the weights of the outgoing carbon dioxide and incoming oxygen for this metabolic mixture may now be calculated by means of the factors: grams urinary N $\times$ 0.84; grams carbohydrate oxidized $\times$ 0.41; grams fat oxidized $\times$ — 0.08. This gives 110 gm. (7.57 + 114.39 — 12.08), for $CO_2 - O_2$. The 24-hour insensible loss of weight was 1220 gm. and the insensible water was therefore 1110 gm. This vapor carried away 644 Calories (1110 $\times$ 0.58).

$$\frac{\text{Heat dissipated by vaporization}}{\text{Total dissipation of heat}} = \frac{644}{2644} = 24.2\%.$$

The same methods were used to determine the percentage of heat lost by vaporization of water by four patients. The first three were youths aged, 15 years, 18 years and 18 years who were convalescent from minimal tuberculosis of the lungs but who were still confined to bed. None of them had fever and they felt well. They lost, respectively, 24.3%, 24.8% and 24.8%.

The last subject, a man 47 years of age, was in the hospital because of Meniere's disease. He had no attacks during the study. The data obtained in this case are especially valuable since he was being underfed and lost 1787 gm. in the 25 days of observation. The calculation was further complicated by a negative nitrogen balance of 1.36 gm. daily. Nevertheless the calculated per cent of heat lost by the vaporization of water was 24.7 even though the metabolic mixture was vastly different from the diet.

The average value for this second group was 24.6%. The response of these subjects all but one of whom remained in bed was very nearly the same as that of the first group who were leading active lives.

The uniformity of performance of these twelve persons indicates that man displays a strong tendency to rid himself by vaporization of water of one-fourth of the heat being produced within his body, provided he is comfortable in regard

to his environment. These studies also show that this tendency is maintained even when there are large differences in the total amount of heat being removed (2200 Calories to 3600 Calories).

However, since we have thus far been dealing with averages of performances that extended over weeks, these data give us no information about the extent of the day to day variation in the percentage of heat removed by vaporization of water. In order to obtain information about this question, we made use of the large respiration chamber described in the preceding paper (Newburgh et al., '37). It was merely necessary to add to its equipment a large balance capable of weighing a human being to 1 gm. The balance was so placed that its pointer could be observed through the window. In order to make a weighing, weights in excess of the weight of the subject were placed on the counterpoise before the chamber was closed. The subject was supplied with a pan of known weight which he held on his lap and to which he added weights until the observer signaled him that balance had been achieved. The subtraction of the weight of the pan and its contents from the weight of the counterpoise gave the weight of the subject.

For 3 or 4 days before entering the chamber the subject received a fixed diet which in some experiments contained much more energy than he required; at other times less; and often was close to the maintenance requirement. The carbohydrate also varied widely in different experiments. The diet eaten within the chamber followed this same plan. Our purpose was to learn what effect these shifts would have on the oxidation of carbohydrate.

The subject entered the chamber the night before an experiment was to be performed. He was supplied with liquid and solid food in appropriate tight containers, all of which had been previously weighed as a single item. At the end of the period, the containers were weighed again to obtain the weight of the ingesta. Urine and feces were voided into weighed containers with tight covers. We were thus able to obtain

an accurate statement of the insensible loss of weight. When all was ready, the chamber was closed and the ventilating pump started. The next morning while the subject was still in bed, the spirometers were washed with chamber air, samples of air from the chamber collected and the ventilation stopped long enough to read the meters. Then the ventilation was begun again, the spirometers opened and the time noted. Next the subject was notified to arise, urinate and be weighed. The time at the end of the weighing was noted. All of the urine up to this time was voided into a separate container and not included in the period. The subject now occupied himself as he pleased during the day. The next morning while the subject was still in bed, samples of air were withdrawn from the chamber. Then the spirometers were closed, the ventilation stopped and the time recorded, simultaneously. The meter readings were made. Immediately thereafter the subject arose, voided and was weighed. The time of the weighing was noted. Samples were withdrawn from the spirometers for analysis.

In addition to obtaining the insensible loss of weight we determined the production of carbon dioxide, the absorption of oxygen and the urinary excretion of nitrogen. This permitted us to calculate the weight of the water vapor by means of the equation:

$$\text{I.W.} = \text{I.L.} - (CO_2 - O_2);$$

and the heat lost by vaporization of water (I.W. $\times$ 0.58).

From the carbon dioxide, oxygen and urinary nitrogen we calculated the heat production by the standard procedure. Since we wanted to compare the heat removed by vaporization with the total dissipation of heat, it was necessary to subtract the heat required to bring the cold food to body temperature from the heat production before applying the formula

$$\frac{\text{I.W.} \times 0.58}{\text{Total Heat Elimination}}$$

This was accomplished by having the subject record the volume and the temperature of fluids whenever he drank them.

Next we compared the heat production determined by indirect calorimetry with the value derived from the insensible loss of weight. To make this latter calculation the composition of the metabolic mixture was ascertained. The urinary nitrogen was used as a measure of the protein. It was assumed that the carbohydrate oxidized equalled the carbohydrate of the diet. The fat of the metabolic mixture was calculated by means of an equation suggested by Lavietes ('35). Its derivation follows:

$$\text{I.L.} = \text{I.W.} + \text{CO}_2 - \text{O}_2$$

$$\text{I.W.} \times 0.58 \times \frac{100}{25} = \text{Calories}$$

$$0.58 \times \frac{100}{25} = 2.32$$

$$\text{I.W.} = \frac{\text{Calories}}{2.32}$$

Calories $= 4.1\ \text{C} + 26.5\ \text{N} + 9.3\ \text{F}$, where C is carbohydrate, N is urinary nitrogen and F is fat.

$$(\text{CO}_2 - \text{O}_2) = 0.41\ \text{C} + 0.84\ \text{N} - 0.08\ \text{F}^{*}$$

$$\text{I.L.} = \frac{4.1\ \text{C} + 26.5\ \text{N} + 9.3\ \text{F}}{2.32} + 0.41\ \text{C} + 0.84\ \text{N} - 0.08\ \text{F}$$

$$\text{I.L.} = 1.77\ \text{C} + 11.42\ \text{N} + 4.009\ \text{F} + 0.41\ \text{C} + 0.84\ \text{N} - 0.08\ \text{F}$$

$$\text{I.L.} = 2.18\ \text{C} + 12.26\ \text{N} + 3.93\ \text{F}$$

$$\text{F} = \frac{\text{I.L.} - (2.18\ \text{C} + 12.26\ \text{N})}{3.93}$$

The heat produced by the oxidation of protein, carbohydrate and fat was determined by multiplying them by their calorific values. Since I.L. had been lessened by a value corresponding to the heat transferred to the cold food, the appropriate number of calories was added to the heat of the metabolic mixture.

Finally we calculated how many grams of carbohydrate had been oxidized so that we could compare that value with the dietary carbohydrate.

We performed thirty-nine experiments on ten young men and women. Each subject was studied two or more times. One of them (O.M.) was in the third trimester of normal pregnancy. The remainder were presumably healthy persons leading active lives. The significant data are presented in tables 2 and 3. Four of the thirty-nine experiments were

* See page 209.

TABLE 2

*Data with which heat production was calculated by indirect calorimetry and from insensible loss of weight*

| SUBJECT | DATE | $O_2$ | $CO_2$ | URINE N | NON-PROTEIN R.Q. | I.L. | CARBO-HYDRATE FED | HEAT TRANS-FERRED TO FOOD |
|---|---|---|---|---|---|---|---|---|
| | | *liters* | *liters* | *gm.* | | *gm.* | *gm.* | *calories* |
| B.DeV., M., age 25 years, height 178 cm., weight 69 kg. | 1–14–35 | 375.7 | 292.0 | 10.2 | 0.772 | 757 | 161 | 41 |
| | 2–24–35 | 370.9 | 276.3 | 13.1 | 0.730 | 741 | 54 | 35 |
| | 3–24–35 | 440.1 | 327.1 | 16.5 | 0.725 | 817 | 96 | 38 |
| | 3–31–35 | 435.9 | 352.8 | 16.1 | 0.812 | 858 | 96 | 40 |
| | 4–30–35 | 379.6 | 288.4 | 11.8 | 0.750 | 667 | 91 | 45 |
| | 5– 1–35 | 399.3 | 303.3 | 11.2 | 0.751 | 687 | 91 | 41 |
| | 11–24–35 | 408.3 | 321.7 | 12.4 | 0.785 | 820 | 183 | 39 |
| | 11–28–35 | 381.3 | 323.0 | 12.5 | 0.857 | 807 | 183 | 40 |
| | 12– 8–35 | 404.6 | 329.0 | 11.8 | 0.816 | 893 | 85 | 42 |
| | 12–23–35 | 469.1 | 385.0 | 11.1 | 0.824 | 981 | 201 | 53 |
| | 2– 2–36 | 436.3 | 365.0 | 15.3 | 0.846 | 954 | 198 | 47 |
| | 2– 4–36 | 514.2 | 423.4 | 26.4 | 0.833 | 1097 | 50 | 55 |
| | 3– 6–36 | 554.7 | 423.3 | 13.9 | 0.756 | 989 | 53 | 30 |
| | 3–29–36 | 447.9 | 349.4 | 10.5 | 0.777 | 837 | 201 | 40 |
| McQ., F., age 25 years, height 163 cm., weight 60 kg. | 2–28–35 | 334.4 | 259.7 | 14.0 | 0.769 | 756 | 52 | 43 |
| | 3–21–35 | 372.2 | 271.5 | 15.1 | 0.707 | 675 | 78 | 56 |
| Wm.M., M., age 30 years, height 177 cm., weight 70 kg. | 3–10–35 | 445.7 | 339.6 | 15.7 | 0.752 | 1292 | 100 | 60 |
| | 4–22–35 | 455.4 | 332.9 | 11.9 | 0.718 | 926 | 95 | 63 |
| | 12–26–35 | 531.0 | 399.7 | 16.4 | 0.742 | 1115 | 53 | 52 |
| | 1– 2–36 | 495.6 | 388.9 | 11.7 | 0.782 | 1101 | 201 | 70 |
| | 1–12–36 | 526.2 | 422.0 | 13.0 | 0.802 | 1123 | 190 | 70 |
| J.S., M., age 29 years, height 168 cm., weight 64 kg. | 10–10–35 | 418.2 | 311.9 | 12.6 | 0.733 | 952 | 59 | 32 |
| | 11– 6–35 | 425.1 | 317.3 | 13.0 | 0.734 | 907 | 89 | 39 |
| | 11–12–35 | 396.8 | 325.2 | 12.5 | 0.824 | 870 | 183 | 42 |
| | 11–19–35 | 404.0 | 331.0 | 12.3 | 0.823 | 842 | 183 | 41 |
| R.L.G., M., age 29 years, height 174 cm., weight 69 kg. | 11–21–35 | 442.8 | 360.4 | 11.7 | 0.816 | 1086 | 183 | 49 |
| | 12– 5–35 | 466.6 | 373.9 | 13.0 | 0.801 | 1084 | 225 | 49 |
| | 12–12–35 | 443.6 | 333.2 | 14.3 | 0.739 | 1015 | 56 | 50 |
| | 12–19–36 | 476.7 | 358.5 | 15.9 | 0.740 | 1015 | 53 | 46 |
| | 1–30–36 | 430.8 | 365.7 | 12.0 | 0.858 | 1138 | 239 | 52 |
| H.A., M., age 23 years, height 180 cm., weight 62 kg. | 10–24–35 | 406.7 | 293.1 | 12.8 | 0.702 | 907 | 59 | 25 |
| | 3– 4–36 | 393.4 | 309.2 | 14.8 | 0.782 | 838 | 117 | 21 |
| L.W.P., F., age 27 years, height 156 cm., weight 55 kg. | 2– 9–36 | 366.7 | 294.9 | 9.5 | 0.803 | 809 | 60 | 31 |
| | 3– 1–36 | 352.0 | 272.0 | 11.0 | 0.766 | 728 | 60 | 27 |
| M.B., F., age 29 years, height 153 cm., weight 45 kg. | 1–15–36 | 281.5 | 232.7 | 8.9 | 0.832 | 672 | 54 | 29 |
| | 1–22–36 | 300.5 | 243.5 | 7.2 | 0.812 | 589 | 61 | 36 |
| O.M., F., age 17 years, height 156 cm., weight 53 kg. | 1–19–36 | 327.1 | 297.7 | 10.6 | 0.936 | 722 | 230 | 60 |
| | 1–24–36 | 345.9 | 288.2 | 10.7 | 0.840 | 778 | 205 | 60 |
| D.S.R., F., age 29 years, height 156 cm., weight 46 kg. | 2–18–36 | 304.6 | 241.3 | 9.2 | 0.790 | 697 | 61 | 25 |
| | 2–26–36 | 306.3 | 241.5 | 9.6 | 0.785 | 556 | 61 | 25 |

TABLE 3

*Per cent of total heat lost by vaporisation in the chamber and comparison of the calculation of heat production from insensible loss of weight with indirect calorimetry*

| | SUBJECT DATE | DIET | | | | CARBOHYDRATE OXIDISED | HEAT LOST BY VAPORISATION, PER CENT OF TOTAL | HEAT PRODUCTION | | DIFFERENCE BETWEEN 9 AND 10 | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Preparatory | | In chamber | | | | Indirect calorimetry | From I.L. | | |
| | | Calories | Carbohydrate | Calories | Carbohydrate | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| | | | *gm.* | | *gm.* | *gm.* | | *Calories* | *Calories* | % | |
| | B.DeV. | | | | | | | | | | |
| 1 | 1–14–35 | 2006 | 161 | 2006 | 161 | 88 | 24.2 | 1772 | 1636 | — 7.7 | |
| 2 | 2–24 | 2000 | 54 | 2000 | 54 | 29 | 24.5 | 1729 | 1696 | — 1.9 | |
| 3 | 3–24 | 2450 | 98 | 2250 | 96 | 27 | 23.2 | 2059 | 1827 | —11.3 | |
| 4 | 3–31 | 2456 | 98 | 1900 | 96 | 154 | 22.6 | 2065 | 1928 | — 6.5 | |
| 5 | 4–30 | 2250 | 94 | 1602 | 91 | 57 | 21.5 | 1782 | | | Cold 3 hours; relative humidity 71% |
| 6 | 5– 1 | 1602 | 91 | 1602 | 91 | 63 | 21.5 | 1874 | | | Relative humidity 71% |
| 7 | 11–24 | 2912 | 189 | 1845 | 183 | 112 | 23.7 | 1929 | 1755 | — 9.0 | |
| 8 | 11–28 | 3005 | 396 | 1845 | 183 | 199 | 23.3 | 1828 | 1725 | — 5.6 | |
| 9 | 12– 8 | 3005 | 396 | 1850 | 85 | 154 | 25.4 | 1925 | 2035 | + 5.4 | |
| 10 | 12–23 | 1906 | 201 | 1906 | 201 | 203 | 23.7 | 2242 | 2134 | — 4.7 | Two quiet preliminary days; I.L.'s 933, 948, 981 |
| 11 | 2– 2–36 | 2374 | 198 | 2374 | 198 | 189 | 24.6 | 2079 | 2054 | — 1.2 | |
| 12 | 2– 4 | 2374 | 198 | 3034 | 50 | 194 (†)[1] | 24.4 | 2430 | 2531 | + 4.2 | |
| 13 | 3– 6 | 2742 | 53 | 2742 | 53 | 100 | 21.4 | 2610 | | | Cold during night |
| Average, omitting nos. 5, 6, 13 | | | | | | | 24.0 | | | — 3.8 | |
| | McQ. | | | | | | | | | | |
| 1 | 2–28–35 | 1793 | 54 | 1566 | 52 | 67 | 27.7 | 1567 | 1743 | +11.2 | |
| 2 | 3–21 | 2009 | 81 | 1758 | 78 | 3 | 23.4 | 1725 | 1537 | —10.9 | |
| Average | | | | | | | 25.6 | | | + 0.2 | |
| | Wm.M. | | | | | | | | | | |
| 1 | 3–10–35 | 2526 | 102 | 2065 | 100 | 68 | 36.2 | 2087 | | | Sensible perspiration; relative humidity 60% |
| 2 | 4–22 | 2627 | 101 | 1865 | 95 | 18 | 26.0 | 2124 | 2124 | 0.0 | Relative humidity 60% |
| 3 | 12–26 | 3260 | 60 | 2134 | 53 | 65 | 26.0 | 2486 | 2596 | + 4.2 | |
| 4 | 1– 2–36 | 3204 | 207 | 2213 | 201 | 137 | 26.7 | 2346 | 2434 | + 3.8 | |
| 5 | 1–12 | 3531 | 196 | 2386 | 190 | 185 | 25.0 | 2501 | 2493 | — 0.3 | |
| Average, omitting no. 1 | | | | | | | 25.7 | | | + 1.9 | |
| | J.S. | | | | | | | | | | |
| 1 | 10–10–35 | 2251 | 94 | 1431 | 59 | 38 | 27.6 | 1935 | 2196 | +13.5 | Relative humidity 59% |
| 2 | 11– 6 | 2987 | 200 | 1844 | 89 | 40 | 26.6 | 1987 | 2059 | + 3.1 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1–12 | 3531 | 196 | 2386 | 190 | 185 | 25.0 | 2501 | 2493 | — 0.3 | |
| Average, omitting no. 1 | | | | | | | 25.7 | | | + 1.2 | |
| | J.E. | | | | | | | | | | |
| 1 | 10–10–35 | 2251 | 94 | 1431 | 59 | 38 | 27.6 | 1935 | 2196 | +13.5 | Relative humidity 59% |
| 2 | 11– 6 | 2987 | 200 | 1844 | 89 | 40 | 26.6 | 1987 | 2059 | + 3.1 | |
| 3 | 11–12 | 2952 | 190 | 1890 | 183 | 162 | 25.1 | 1889 | 1876 | — 0.7 | |
| 4 | 11–19 | 2915 | 189 | 1884 | 183 | 165 | 23.7 | 1924 | 1808 | — 6.0 | |
| Average | | | | | | | 25.8 | | | + 2.5 | |
| | R.L.G. | | | | | | | | | | |
| 1 | 11–21–35 | 2912 | 189 | 1884 | 183 | 175 | 28.5 | 2110 | 2387 | +13.1 | |
| 2 | 12– 5 | 3340 | 303 | 2118 | 225 | 158 | 27.2 | 2215 | 2344 | + 5.8 | |
| 3 | 12–12 | 2969 | 60 | 2090 | 56 | 49 | 28.8 | 2054 | 2358 | +14.8 | |
| 4 | 12–19 | 3259 | 60 | 2134 | 53 | 54 | 26.4 | 2229 | 2353 | + 5.6 | |
| 5 | 1–30–36 | 3210 | 243 | 2158 | 239 | 234 | 29.8 | 2070 | 2461 | +18.9 | |
| Average | | | | | | | 28.1 | | | +11.6 | |
| | H.A. | | | | | | | | | | |
| 1 | 10–24–35 | 2532 | 101 | 1422 | 59 | 0 | 28.3 | 1889 | | | Too warm |
| 2 | 3– 4–36 | 3015 | 123 | 2022 | 117 | 99 | 25.1 | 1852 | 1844 | — 0.4 | |
| Average, omitting no. 1 | | | | | | | 25.1 | | | — 0.4 | |
| | L.W.P. | | | | | | | | | | |
| 1 | 2– 9–36 | 828 | 60 | 828 | 60 | 128 | 25.5 | 1743 | 1858 | + 6.6 | |
| 2 | 3– 1 | 828 | 60 | 828 | 60 | 73 | 24.8 | 1656 | 1658 | + 0.1 | |
| Average | | | | | | | 25.2 | | | + 3.3 | |
| | M.B. | | | | | | | | | | |
| 1 | 1–15–36 | 2004 | 64 | 942 | 54 | 123 | 27.3 | 1343 | 1539 | +14.6 | |
| 2 | 1–22 | 2000 | 64 | 1400 | 61 | 117 | 22.7 | 1415 | 1348 | — 4.7 | |
| Average | | | | | | | 25.0 | | | + 5.0 | |
| | O.M. | | | | | | | | | | |
| 1 | 1–19–36 | 2085 | 230 | 2085 | 230 | 260 | 23.4 | 1594 | 1486 | — 6.0 | |
| 2 | 1–24 | 2085 | 230 | 1753 | 205 | 162 | 25.7 | 1654 | 1656 | + 0.1 | |
| Average | | | | | | | 24.6 | | | — 3.0 | |
| | D.S.R. | | | | | | | | | | |
| 1 | 2–18–36 | 2004 | 64 | 1400 | 61 | 90 | 27.0 | 1441 | 1586 | +10.3 | |
| 2 | 2–26 | 2004 | 64 | 1400 | 61 | 84 | 21.2 | 1447 | 1252 | —13.4 | |
| Average | | | | | | | 24.1 | | | — 1.6 | |

[1] This experiment was calculated in the standard manner, but it is probable that the carbohydrate oxidized is incorrect since the subject was grossly overfed and may have converted some carbohydrate to fat.

unsuccessful because the subjects were uncomfortably cold or warm. A fifth one is excluded because the relative humidity was so great that it presumably hindered the evaporation of water.

The remaining thirty-four experiments reveal a variation in the per cent of heat removed by vaporization of water from 21.2 to 29.8. This degree of inconstancy in the percentage of heat vaporized by persons residing in the chamber, raises the question whether the conditions imposed by it are in part responsible for the variation. Demonstrable evidence of an emotional disturbance associated with confinement in the chamber was recorded in the case of R.L.G. He had been one of the subjects of the earlier studies already described (Newburgh, Wiley and Lashmet, '31). Under those conditions when his activities were not hampered, he removed 25.2% of the heat by vaporization. But in the chamber, the results of the five periods ran from 26.4% to 29.8%. These high values were accompanied by a diuresis and a sharp loss of weight even though he was adequately fed. Since the response of this subject was in sharp contrast to that of all the others, we have excluded him when dealing with the averages cited below.

The variation exhibited by the nine other subjects was from 21.2% to 27.7%. The average value is 25.1%.

When the means of all the periods for each of the nine subjects are examined it is found that they all fall within the limits of 24.0% and 25.8%. The close agreement of the average performances of the nine subjects emphasizes the desirability of using the mean of several 24-hour periods whenever heat production is calculated from insensible loss of weight.

In that calculation it is assumed that the individual has lost 25% of the heat by vaporization. Hence an actual loss by vaporization of 24 or 26% would cause an error of $\pm 4\%$ in the final calculation.

The calculation of total heat from insensible loss of weight is also exposed to a second error since it is assumed that the carbohydrate oxidized is the same as the carbohydrate fed.

The significance of this potential error may be judged by means of the following considerations. When solving the equation,

$$\text{I.W.} = \text{I.L.} - (CO_2 - O_2)$$

the numerical value of $(CO_2 - O_2)$ is in part obtained by multiplying the dietary carbohydrate by 0.41. Accordingly I.W. is lessened by 0.41 per gram of carbohydrate assumed to have been oxidized. The effect upon the heat production calculated from I.L. is, therefore, $0.41 \times 0.58 \times \frac{100}{25} = 0.95$ calorie per gram difference between the carbohydrate ingested and oxidized. Our conception of the relationship between the ingestion and oxidation of carbohydrate is this. The individual whose liver glycogen is depleted will store large amounts of the ingested carbohydrate in the liver, even if the inflow is small. A person who possesses an unusually large store of liver glycogen may convert some of it to glucose as a source of energy even if the ingestion of carbohydrate is liberal. The actual amounts of glycogen lost or gained by the liver will be importantly influenced by the expenditure of energy and by the calories of the diet. An individual who expends approximately the same amount of energy daily and who takes a fixed diet will come into carbohydrate balance within a few days and remain so as long as the conditions persist. This last condition could only be established for the chamber experiments by restricting the subject's activity for some days before he entered the chamber and to feed a fixed diet throughout. We have only one experiment of this kind, that is no. 10 for the subject B. DeV. It will be seen that he did in fact oxidize the carbohydrate of the diet while in the chamber, as closely as it can be calculated.

In the remainder of the experiments, the activity of the subjects was in no way restricted during the preliminary days. Under these circumstances, none of the dietary plans that were tried could be relied upon to establish carbohydrate balance during the 24 hours in the chamber.

These experiments make it clear that the calculation of heat production from a single 24-hourly insensible loss of weight, cannot be depended upon to give a precise answer. Such a single determination does, nevertheless, yield a first approximation which we have found useful in the formulation of dietary plans. When, however, the average of the 24-hourly losses for each subject (omitting R.L.G.) is the basis of the calculation, the values obtained for the heat production by this method differed less than 5% from the figure determined by indirect calorimetry.

The data that we have reported thus far were obtained from normal subjects or patients whose regulation of heat was presumably normal. It will be interesting to learn whether edema, anaemia or heart failure causes a shift in the percentage of heat removed by vaporization. Clearly the method will give unsatisfactory results when the internal temperature is rising or falling. Several investigators have thought that rapid large shifts in the water content of the body are reflected in a change in the percentage of heat lost by vaporization. We have produced dehydration to the extent of 6% of the body water without causing a shift in the percentage of heat lost by vaporization (Newburgh and Johnston, '34).

We do not offer this method of measuring heat production as a substitute for either the calorimeter or the respiration chamber but rather as a means of extending the range of measurement. The calculation of heat from insensible loss may be satisfactorily carried out in individuals who continue to lead their customary lives provided they do not indulge in strenuous exercise or heavy manual labor. The method was successful in the case of the two graduate students in chemistry who produced approximately 3500 Calories daily. The second advantage of measuring heat in this way is that it permits one to obtain a continuous record for as many consecutive days as is desired. The simplicity of the equipment required is a third advantage.

Lavietes ('35), who has published a critique of the method, makes the following statement:

That Newburgh's experiments show remarkable agreement between actual and predicted metabolism over long periods of time can not be doubted. Under restricted conditions the insensible perspiration may yield valuable information concerning the energy metabolism which can be obtained otherwise in no way short of direct calorimetry. It seems extremely doubtful, however, . . . . that the method is applicable during conditions of changing hydration, or of value in the study of edema or diuresis.

Lavietes' distrust of the method when body fluids are shifting rapidly is largely based on the unsatisfactory results obtained by him. For example, he obtained his own 48-hourly insensible losses for 20 days while he pursued his ordinary activities. From the eighth through the thirteenth day he took 40 gm. of urea daily. The average of the losses during the three corresponding periods was 11% less than the average of the preceding and subsequent ones. Since Lavietes believes that the heat production was not diminished to a corresponding degree, he assumes that the method failed. Such a conclusion is extremely hazardous since he had no actual measurement of his heat production at any time. The following data indicate how greatly small movements of which one is not aware, influence the total heat production. In eleven 24-hourly periods, the heat production of a highly trained subject living in the respiration chamber, determined by indirect calorimetry, varied from 1729 Calories to 2242 Calories. The subject, questioned at the end of each period, was quite unable to predict the outcome.

## SUMMARY

The results of this study are based upon observations made upon twenty different individuals. Most of them were young normal adults. The remainder were patients whose regulation of heat was presumably normal. Nine of them were studied in detail in the respiration chamber; four others while they remained in bed, and the remainder while they were pursuing active lives.

The chamber permitted us to measure the percentage of heat removed by vaporization during 24 hours. There are twenty-nine such periods (omitting R.L.G.) from nine subjects. The lowest per cent was 21, the highest 28. The average of the twenty-nine periods was 25%. In the two earlier series of experiments, the conditions permitted only a determination of the average per cent of heat removed by vaporization. Here the least was 23.8% and the greatest 25.2%. The average was 24.5%. The grand average for the whole series is 24.7%. Since biological variation is evident, we have adopted the nearest whole number which is 25%.

This narrow range is ample support for the belief that man possesses a mechanism designed to rid him by vaporization of water of a fixed percentage of the heat produced within his body. Conditions that cause him to feel uncomfortably warm or cool will disturb this relationship. The discomfort is the signal to adjust himself to the environment. He strives to do this by lessening or increasing the barrier between himself and the environment, by changing the rate of his activity or by making the surroundings more suitable.

This mechanism has been used as a basis for the calculation of the heat produced by normal human beings whose activity varied from continuous residence in bed to that of the busy laboratory worker. It is evident from the experiments in the chamber that the percentage of heat removed by vaporization of water during a single 24 hours was not sufficiently fixed to afford a reliable determination of the total heat. However, when the average of several periods was used, the maximal errors (omitting R.L.G.) attributable to variability in the per cent of heat lost by vaporization was only $\pm 5\%$.

The second potential error in this calculation is due to the assumption that the carbohydrate oxidized is the same as the dietary carbohydrate. This error may be reduced to a negligible quantity by 1) feeding a fixed diet; 2) establishing a fixed plane of activity; 3) discarding the first few days of the study; 4) using the average of several consecutive days following the period of adjustment.

The calculation of heat production from the insensible loss of weight, the carbohydrate of the diet and the urinary nitrogen by the technique described above yielded values that differed less than 5% from those obtained by indirect calorimetry, when averages of several 24-hourly periods were used.

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# IMPROVED GROWTH IN RATS ON IODINE DEFICIENT DIETS

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In previously reported work of this laboratory on the results of iodine deficiency in the rat (Remington and Levine, '36), the low iodine diet employed has been as follows: wheat gluten 20, yellow corn meal[1] 76, calcium carbonate 3, sodium chloride 1, irradiated yeast 0.2. Young rats maintained for 35 days on this diet show the following changes in the thyroid: 1) enlargement, 2) hyperplasia, 3) edema, 4) almost complete loss of colloid, and 5) marked loss of iodine. Since these changes are fully prevented by the addition of iodine (1 to 2 γ per day) either as iodides or certain iodine-bearing foods (Remington, Coulson and Levine, '36) they are considered as specific consequences of lack of this element.

This diet falls far short of satisfying the ideal for the study of deficiencies, i.e., full adequacy in all known essentials except the one being investigated. Glaringly evident are the unbalance between Ca and P, and the deficiency in vitamins of the G($B_2$) group. Probable deficiencies also exist in inorganic elements, and in the quality of the protein. It does not support normal growth. Rats 4 weeks of age, averaging 60 gm. in weight, when placed on the diet for 5 weeks, with or without added iodide, grow only to 110 to 130 gm., as compared with 180 to 200 gm. (average for both sexes) for rats on our colony ration. Attempts to rear rats on this diet to full size and sexual maturity have not been successful.

[1] Whole yellow corn meal as sold for chick feeding.

Obviously, if one desires to answer such questions as to whether or not normal growth can be obtained in iodine deficiency, or what is the effect of deficiency continued into adult life on the animals themselves, on the composition and histology of the thyroid, or on reproduction, the first step must be an attempt to improve the diet without notably decreasing its goiter-producing power. Many other problems affecting the utilization of iodine in the rat can be attacked in a quantitative way, if such a diet is possible. Difficulties have been the universal presence of traces of iodine in natural foods or concentrates prepared from them, and the presence of significant traces in many chemically pure salts. It should be borne in mind that a degree of contamination which will augment the iodine intake of the rat 0.1 to 0.2 γ per day can produce a detectable difference in the size of the thyroid and its iodine content. For instance, if a given supplement is to be added to the diet at a level of 2%, it should not contain more than 0.00005% (500 p.p.b.) iodine, since this amount would add approximately 0.1 γ per day to that unavoidably present in the basal diet. It is, on the other hand, quite probable that if a given supplement improves growth, and the economy of food utilization for growth, the daily intake of iodine necessary to maintain a given degree of thyroid abnormality may be altered, in which case our ideas as to iodine requirement (Levine, Remington and von Kolnitz, '33) will require revision, since these were developed on rats which made subnormal growth.

## EXPERIMENTAL

The first step in improving the diet was to counteract its rickets-producing property by reducing the proportion of $CaCO_3$ from 3 to 1%, it having been shown that this change did not lessen the degree of thyroid enlargement. This change permitted the omission of irradiated yeast. The new basal diet (no. 327) is as follows: wheat gluten 20, yellow corn meal 78, $CaCO_3$ 1, NaCl 1. Experiments were then made in supplementing the diet in three ways, viz., 1) improvement in protein quality by substituting purified (vitamin-free) casein

for part or all of the wheat gluten; 2) improvement of vitamin and mineral supply by addition of small percentages (0.5 to 4%) of dried pig liver, or 3) by the addition of similar small percentages of dried brewers yeast.

The handling of the animals was essentially the same as that used in earlier experiments. Young rats, approximately 28 days of age and weighing around 60 gm., were placed on screens and maintained on the diets with distilled water for 35 days, after which they were killed by chloroform and the thyroids removed. Records were kept of the gain in weight and of the food consumed. Ten rats, either five of each sex or four of one and six of the other, were placed on each diet.

The glands on removal were placed in special nickel boats, and weighed in a stoppered weighing bottle. All of the glands from a given group were placed in the same boat, which was then dried to constant weight at 100°, for calculation of dry matter. Iodine was determined colorimetrically on the entire contents of the boat by the method of the writer (Remington, McClendon, von Kolnitz and Culp, '30).

### *Effect of casein*

Results obtained when vitamin-free casein was substituted for part or all of the wheat gluten are shown in table 1. Five per cent of casein, substituted for wheat gluten, produces a significant gain in rate of growth, which still is only about half that of normal rats. Further increases in proportion of casein do not bring about any greater gains. When the thyroid is considered, however, it appears that with increasing proportions of casein there is a progressive decrease in thyroid weight, and an increase in dry matter and iodine content, the data conforming well to our curves (Remington and Levine, '36). Either the casein carries a significant amount of iodine, or its presence in the diet makes possible a higher degree of storage in the thyroid of the small amount of the element available in the diet. The effects observed can be attributed to increased iodine supply if the casein contains 200 to 300 parts per billion, a point which will be discussed later.

### *Effect of liver*

Fresh pig liver was obtained from a distributor, sliced, partially dried on a steam table, ground through a meat chopper, the drying completed in an oven at 80°, then reground and stored in tightly sealed jars. The first series of experiments was run parallel to the casein experiments, with

TABLE 1

*Effect of casein*

| DIET | 327 | 328 | 329 | 330 | COLONY |
|---|---|---|---|---|---|
| Wheat gluten | 20 | 15 | 10 | 0 | |
| Casein | 0 | 5 | 10 | 20 | Commercial |
| Corn meal | 78 | 78 | 78 | 78 | dog |
| $CaCO_3$ | 1 | 1 | 1 | 1 | biscuit |
| NaCl | 1 | 1 | 1 | 1 | |
| Number of rats | 10 | 10 | 10 | 10 | 10 |
| Sex | 4 M–6 F | 4 M–6 F | 5 M–5 F | 3 M–7 F | 5 M–5 F |
| Gain in weight (gm.) | 51±2 | 68±4 | 74±4 | 67±4 | 120 |
| Thyroid weight (mg.) | 27.4 (19.1–37.7) | 24.9 (18.2–36.1) | 23.1 (18.2–27.9) | 18.3 (15.1–21.1) | 15.8 (11.9–20.2) |
| Thyroid weight per 100 gm. body weight (mg.) | 23.9±1.0 (18.7–35.2) | 18.9±0.6 (13.7–21.9) | 17.1±0.5 (13.6–21.6) | 14.1±0.4 (11.4–16.5) | 8.8±0.4 (6.3–11.7) |
| Dry matter (%) | 21.0 | 23.6 | 25.9 | 27.4 | 32.0 |
| Iodine, dry basis (%) | 0.0137 | 0.0297 | 0.0451 | 0.0595 | 0.2000 |
| Total iodine (γ) | 0.79 | 1.75 | 2.70 | 2.99 | 10.1 |
| Degree of enlargement | 2.4 | 1.9 | 1.7 | 1.4 | |

the same control group. Dried liver was added to the diet at levels of 1.5 and 3%. Both groups showed greater growth than the casein-fed rats, but unlike the latter, these had considerably larger thyroids, of lower iodine content, than the controls. Since work in this direction appeared to show promise, a second series was run (table 2), the liver being fed this time at levels of 0.5, 1.0, 2.0 and 4.0% of the diet, replacing in each case the same amount of gluten. In addition, a group

of rats was fed 2% of dried lean beef (chuck). In this series, maximum growth was obtained at the 2% liver level, as was also maximum degree of thyroid enlargement (3.7 times normal weight). Beef muscle also stimulated growth, but to less than

TABLE 2

*Effect of liver. Series II*

| DIET | 327 | 340 | 341 | 342 | 343 | 344 | COLONY |
|---|---|---|---|---|---|---|---|
| Wheat gluten | 20 | 19.5 | 19 | 18 | 16 | 18 | Commercial dog biscuit |
| Dried liver | 0 | 0.5 | 1 | 2 | 4 | 0 | |
| Corn meal | 78 | 78 | 78 | 78 | 78 | 78 | |
| $CaCO_3$ | 1 | 1 | 1 | 1 | 1 | 1 | |
| NaCl | 1 | 1 | 1 | 1 | 1 | 1 | |
| Dried lean beef | 0 | 0 | 0 | 0 | 0 | 2 | |
| Number of rats | 10 | 10 | 10 | 10 | 10 | 10 | |
| Sex | 6 M–4 F | 6 M–4 F | 6 M–4 F | 6 M–4 F | 6 M–4 F | 6 M–4 F | |
| Gain in weight (gm.) | 66 ± 3 | 81±2 | 92±3 | 104±4 | 105±5 | 85±4 | 125 |
| Thyroid weight (mg.) | 30.4 | 34.0 | 42.3 | 52.1 | 46.1 | 37.3 | 15.8 |
| Thyroid weight per 100 gm. body weight (mg.) | 23.9±1.0 | 23.9±1.0 | 27.8±1.4 | 32.3±1.7 | 28.2±1.1 | 25.6±0.8 | 8.8±0.4 |
| Dry matter (%) | 22.4 | 21.8 | 21.3 | 20.9 | 21.1 | 22.4 | 32.0 |
| Iodine, dry basis (%) | 0.0064 | 0.0049 | 0.0032 | 0.0051 | 0.0034 | 0.0055 | 0.2000 |
| Total iodine (γ) | 0.44 | 0.36 | 0 .29 | 0.55 | 0.33 | 0.46 | 10.1 |
| Degree of enlargement | 2.4 | 2.4 | 2.8 | 3.7 | 3.2 | 2.6 | ..... |

half the degree of liver, and the thyroids were not significantly larger than the controls (2.6 times normal against 2.4). In all cases the iodine content of the thyroid is so low that no importance can be attached to such differences as exist.[2]

[2] The total amount of iodine contained in the dried thyroids of ten animals was 3 to 5 γ, which cannot be estimated by present methods with any higher degree of accuracy than about ± 20%.

### *Effects of yeast*

In the third series (table 3) dried brewers yeast, furnished through the courtesy of Dr. Harold Levine of the Pabst Brewing Company, was added to diet 327 in the proportions of 0.5, 1.0, 2.0 and 4.0% replacing an equal amount of gluten. Growth is improved by the yeast, most marked effect being observed at the 2% level, but no significant changes in thyroid weight, dry matter, or iodine content are observed. Neither was growth as good as with equal additions of liver.

TABLE 3

*Effect of brewers yeast*

| DIET | 327 | 345 | 346 | 347 | 348 |
|---|---|---|---|---|---|
| Wheat gluten | 20 | 19.5 | 19 | 18 | 16 |
| Dried yeast | 0 | 0.5 | 1 | 2 | 4 |
| Corn meal | 78 | 78 | 78 | 78 | 78 |
| $CaCO_3$ | 1 | 1 | 1 | 1 | 1 |
| NaCl | 1 | 1 | 1 | 1 | 1 |
| Number of rats | 10 | 10 | 10 | 10 | 10 |
| Sex | 5 M–5 F | 5 M–5 F | 5 M–5 F | 5 M–5 F | 5 M–5 F |
| Gain in weight (gm.) | 54±2 | 64±4 | 67±3 | 80±4 | 84±4 |
| Thyroid weight (mg.) | 22.1 | 26.1 | 25.6 | 28.1 | 27.9 |
| Thyroid weight per 100 gm. body weight (mg.) | 19.2±0.6 | 18.5±0.8 | 19.9±1.0 | 19.7±0.8 | 19.1±0.5 |
| Dry matter (%) | 24.1 | 25.2 | 24.8 | 24.9 | 24.8 |
| Iodine, dry basis (%) | 0.0084 | 0.0074 | 0.0083 | 0.0067 | 0.0086 |
| Total iodine (γ) | 0.45 | 0.49 | 0.53 | 0.47 | 0.60 |
| Degree of enlargement | 1.9 | 1.9 | 2.0 | 2.0 | 1.9 |

## DISCUSSION

The four supplements fed; casein, liver, beef muscle and yeast, all improved the growth of the animals, but in different amounts. Rats receiving 5% of casein in place of the same amount of wheat gluten gained 17 gm. more than the controls, but this gain was exceeded by those receiving 1% of dried liver, or 2% of beef muscle or yeast. Since casein is a widely used and satisfactory protein in experimental diets, it does not seem likely that the greater improvement in growth resulting from much smaller additions of the other supplements

can be due to protein quality alone. It is possible that the vitamin content, particularly with regard to A and G, of the corn meal, is insufficient for rapid growth, and that this partial deficiency is compensated by the vitamins present in liver or yeast. Deficiency in iron is also a possibility, although rats on the basal diet do not become anemic within the experimental period employed. Even though the present investigation does not reveal specific deficiencies in the basal diet (327) it has achieved the desired end of producing normal, or nearly normal, growth on diets markedly deficient in iodine. An investigation of the effects of augmenting the vitamin content of diet 327 with carotene or flavin would contribute to the specificity of our knowledge as to how the liver acts, and might make possible a further simplification of the problem.

When it comes to the effect on the thyroid gland, i.e., degree of goiter as indicated by the criteria previously proposed: fresh weight, iodine content, and dry matter content of the gland, the response to different supplements is quite different. Casein added to the diet in progressively increasing amounts brought about a diminution in the severity of goiter in almost exact relation to the amount fed. Previous experience with adding iodides or iodine-bearing foods in quantities insufficient for full protection against goiter, enables us to calculate that if the effect is due to iodine in the casein, it should contain between 200 to 300 parts per billion. Two analyses of casein from the same source made several years ago revealed twelve and eighteen parts, respectively, an insignificant amount. It seemed possible therefore that unless casein itself is able to protect the thyroid against the results of iodine lack, either the earlier analysis must have been in error, or the present lot of casein differs in iodine content from previous ones. Accordingly, the particular lot of casein employed was analyzed, the chemist not being informed as to the purpose of the analysis or probable values.[3] Four determinations gave values of 250, 254, 259 and 267 parts per billion, amply con-

[3] Iodine analyses on dietary materials were made by Mr. Cecil L. Smith, on thyroid glands by Mr. E. J. Coulson.

firming the belief that the reduction in degree of goiter obtained with casein is due to iodine contamination, and rendering it unnecessary to postulate any specific protective action due to casein.

The marked increase in severity of goiter when liver is added to the diet was not expected, and is rather disturbing when considered alongside the earlier work of this laboratory in which it appeared that quantitative relationships exist between iodine intake and degree of hyperplasia of the thyroid. The dried pig liver contained about 150 p.p.b. of iodine, enough to contribute 0.04 γ per day per rat when fed at the 2% level. This additional intake of iodine should have had a slight protective action, probably too small to measure. Instead of such an effect, however, the degree of enlargement of the glands of rats receiving 2% liver is 3.7 times normal, compared with 2.4 for those receiving no liver.

It was suggested earlier in the paper that augmented growth might conceivably increase the iodine requirement of the rat, and hence bring about a more severe thyroid condition on the same iodine intake. Fortunately, the yeast series bears on this question. Rats receiving 2% of yeast made the same gain as those receiving 1% of liver, about 26 gm. more than the controls in both cases. The yeast contained less than 20 p.p.b. of iodine, hence its contribution of that element was negligible. Nevertheless yeast did what we had expected the liver to do, increased growth without affecting the degree of thyroid enlargement. If, then, rate of growth can be increased by 50% by the addition of 2% of yeast, which neither increases nor decreases appreciably the iodine content of the ration, and this improved growth is not accompanied by an increased severity of the thyroid condition, we cannot with surety attribute such increased severity to the effect of growth when obtained by means of liver.

To explain the effect of liver on the thyroid, two possibilities are presented: 1) the presence in the liver of some substance, the metabolism of which causes a drain on the thyroid mechanism, or 2) the presence of a specific thyroid-stimulating

hormone. In Marine's early observations on goiter in young brook trout fed largely on chopped liver, he wrote ('14): "It is also probable that the liver contains some substances in excess, in attempting to utilize which the animal exhausts other elements necessary for nutrition which are not present in the liver in sufficient amounts." This view is reflected

TABLE 4

*Economy of food utilization for growth*

| DIET NO. | SUPPLEMENT | TOTAL GAIN | TOTAL FOOD | FOOD FOR GRAM GAIN |
|---|---|---|---|---|
| | | Series I | | |
| 327 | Basal | 51 | 348 | 6.8 |
| 328 | 5% casein | 68 | 335 | 4.9 |
| 329 | 10% casein | 74 | 383 | 5.2 |
| 330 | 20% casein | 67 | 382 | 5.7 |
| 331 | 1.5% liver | 81 | 398 | 4.9 |
| 332 | 3.0% liver | 87 | 417 | 4.8 |
| | | Series II | | |
| 327 | Basal | 66 | 385 | 5.9 |
| 340 | 0.5% liver | 81 | 411 | 5.1 |
| 341 | 1.0% liver | 92 | 437 | 4.8 |
| 342 | 2.0% liver | 104 | 458 | 4.4 |
| 343 | 4.0% liver | 105 | 475 | 4.5 |
| 344 | 2.0% lean beef | 85 | 406 | 4.8 |
| | | Series III | | |
| 327 | Basal | 54 | 317 | 5.9 |
| 342 | 2.0% liver | 99 | 411 | 4.2 |
| 345 | 0.5% yeast | 64 | 332 | 5.2 |
| 346 | 1.0% yeast | 67 | 348 | 5.2 |
| 347 | 2.0% yeast | 80 | 368 | 4.6 |
| 348 | 4.0% yeast | 84 | 346 | 4.1 |
| Colony | | 120 | 482 | 4.0 |

in the first suggested explanation. Marine ('22) also states that "liver, particularly pig liver, was the most potent of a great variety of meats in causing thyroid hyperplasia in dogs and cats . . . ," but since his protocols have not been published, one cannot be sure that he has ruled out differences due to other deficiencies, such as iodine. Marine also believed that increased protein in the diet increases the tendency to goiter, but in the present work we have not appreciably varied

the percentage of protein, hence the experiments do not bear on this point.

All the supplements fed, in addition to improving growth, also increased the economy of food utilization for growth, as shown in table 4. If we disregard the small amount of fat contained in the liver and yeast, the caloric value of all diets was the same. The most efficient utilization of food was found in rats fed 2% liver or 4% yeast: 4.2 and 4.1 gm. of food, respectively, for each gram of gain. On the basal diet alone it required 5.9 gm., while a typical group on the colony diet (dog biscuit) consumed only 4 gm. of food for each gram of gain.

From the foregoing it appears that diet 342, containing 2% of dried pig liver, should enable us to reach the desired end of prolonging our experimental period through a normal life span of the animals. Rats have been raised to maturity on this diet, with a progressively greater degree of thyroid hyperplasia (up to 5 months), have produced normal numbers of living young and reared them without loss to weaning age, despite the fact that their thyroids were markedly hyperplastic, contained practically no colloid after 8 weeks, and only 3 to 5% as much iodine as those of rats on colony ration.

## SUMMARY

The subnormal growth of young rats fed the goiter-producing diet of Levine and Remington is improved when a part of the wheat gluten is replaced by 1) purified casein, 2) dried pig liver, or 3) dried brewers yeast. Equal gains were produced with 10% casein, 1% liver or 2% yeast. The addition of 2% liver produced most satisfactory growth.

Purified casein carries sufficient iodine to render it unsuitable for use in goiter-producing diets. Yeast was without effect on degree of goiter but liver aggravated the condition.

A suitable diet for goiter studies on rats is wheat gluten 18, dried pig liver 2, yellow corn meal 78, calcium carbonate 1, sodium chloride 1. On this diet rats reach maturity and produce normal numbers of living young despite almost complete absence of iodine and colloid from the thyroid gland.

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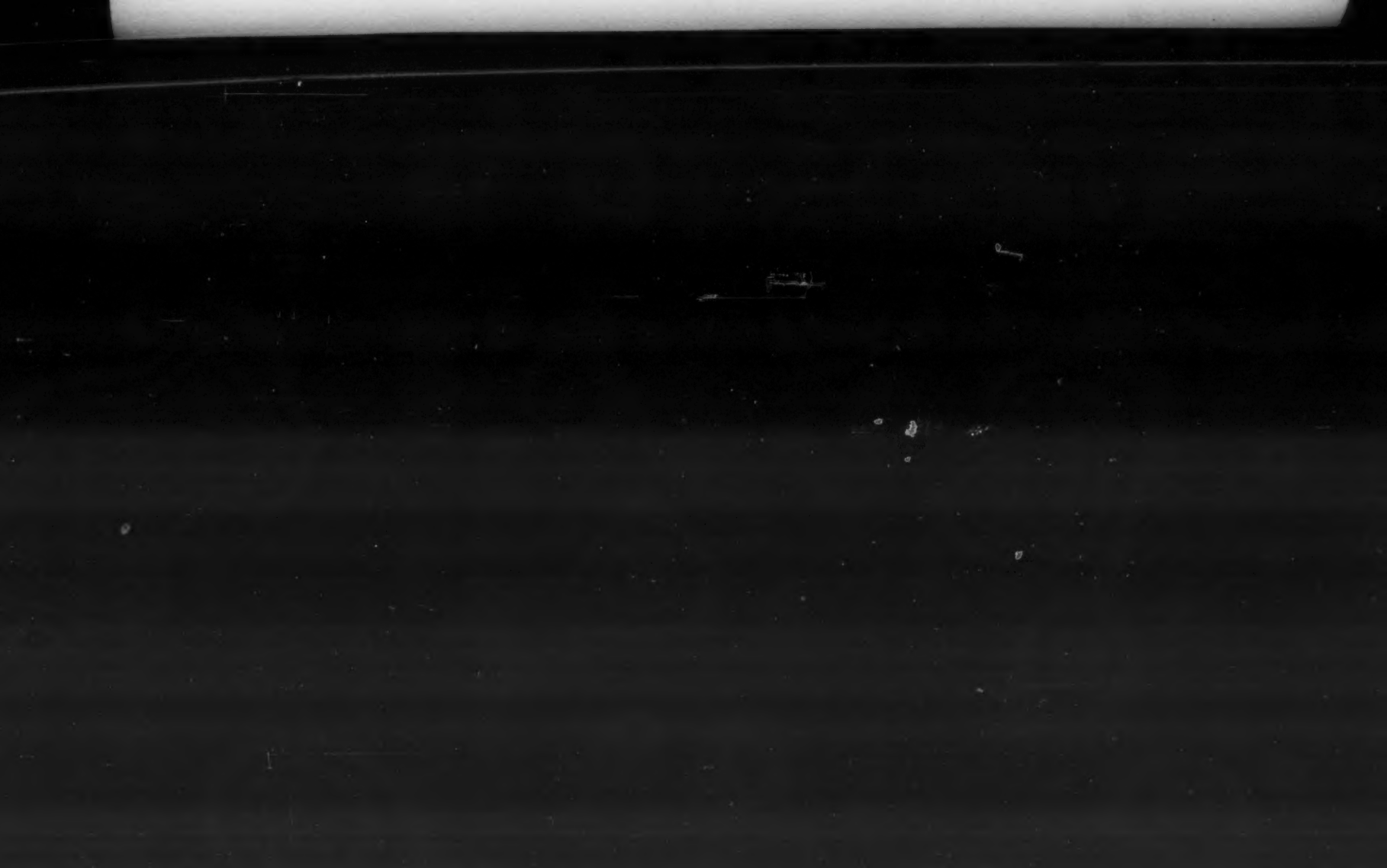

# A STUDY OF THE EFFECT OF VITAMIN B AND IODINE ON THE WEIGHT, IODINE CONTENT AND STRUCTURE OF THE THYROID GLAND OF THE RAT

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FOUR FIGURES

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Cowgill ('32) and Hendricks ('33) have suggested that the vitamin B($B_1$) requirement of an animal is proportional to its metabolism. The thyroid gland, through its iodine containing hormone (thyroxin), has been shown to provide a means both for maintaining a higher rate of metabolism than would otherwise be obtained and for varying the rate of metabolism to meet changing physiologic needs.

It has been shown that the function of the thyroid can be limited by a deficiency of iodine. McClendon ('27) and Orr ('31) have reviewed the subject of goiter and its relation to iodine supply. Studies of the effect on the thyroid of cabbage feeding (Webster and Chesney, '30), of methyl cyanide (Marine, Rosen and Cipra, '33), of calcium (Hellwig, '34) and of chlorides (Hibbard, '33) have indicated that a number of factors may increase the needs of the body for iodine or for the iodine containing hormone and thereby may play a part in the development of simple goiter.

A number of workers have associated thyroid function with the vitamin B complex and lately with the B($B_1$) fraction. McCarrison ('14) noticed hyperplasia of the thyroid of pigeons fed a diet of polished rice. The suggestion has been made (Aalsmeer, '32) that beri-beri is associated with dysfunction of the thyroid. Stepp and György ('27) state that